ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE

Fiscal Year 1975

Volume V

(Parts V and VI)

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service   National Institutes of Health

ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE

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Volume V

(Parts V and VI)

U.S. NATIONAL CANCER INSTITUTE

Report of program activities

Part V - Division of Cancer Treatment

Part VI - Division of Cancer Control and Rehabilitation

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NATIONAL CANCER INSTITUTE
ANNUAL REPORT
July 1, 1974 through June 30, 1975

TABLE OF CONTENTS

DIVISION OF CANCER TREATMENT

DIRECTOR

Page

Introduction i

Code for Division of Cancer Treatment Program Logic 1

Table I Analysis of Contract Activities 3

Table II Analysis of Contracts by Activity 5

Table III Analysis of Activities by Contract 37

Table IV Description Contracts 79

Project Reports:

CM-06401 Data Processing Systems for Scientific Management Information in the Division of Cancer Treatment 138

CM-06402 Tumor Growth Kinetics and Chemotherapy 141

CM-07025 Biomedical Engineering for Intramural Research,DCT 148

CM-07085 Quantitative Studies on Tumor Cell Kinetics and Biomarker Synthesis; and on the Dynamics of Cell Kinetic Parameters and Implications for Cancer Therapy 157

CM-09000 Hemopoietic Stem Cells in Human Peripheral Blood 164

ASSOCIATE DIRECTOR FOR DRUG RESEARCH AND DEVELOPMENT

Summary Report 167

Project Report:

CM-07101 Computer Methods for Drug Pre-selection Based on Structure-Activity 175

Mammalian Genetics and Animal Production Section 179

Publications Section 185

Page

Drug Development Branch

Summary Report 191

Chemical and Drug Procurement Section 213

Chemical and Drug Records Section 225

Clinical Drug Distribution Section 229

Project Report:

CM-03584 Research in Dosage Form Development of New Antitumor Drugs 235

Drug Design and Chemistry Section 239

Project Reports:

CM-03580 Chemical Research in the Development of New Anticancer Drugs 241

CM-03581 The Analytical Chemistry of New Anticancer Drugs 245

CM-03582 Structure-Activity Studies of Anticancer Drugs 247

Data Processing and Computer Systems Development Section 251

Natural Products Section 253

Scientific Records Section 261

Drug Evaluation Branch

Summary Report 265

Automated Information Section 281

Biochemistry Section 285

Molecular Biology and Methods Development Section 289

Project Reports:

CM-03579 Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression 294

CM-03583 Modification of Cell Surfaces to Limit Cell Division and Increase Immunogenicity 296

Screening Section 299

Laboratory of Experimental Chemotherapy

Page

Summary Report 311

Project Reports:

CM-03575 Chemotherapy and Combined Modality Investigations of Antitumor Agents 312

CM-03577 Immunochemotherapy Studies in Various Tumor Systems 323

CM-07100 Tumor Biology and Chemotherapy 328

ASSOCIATE DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

Summary Report 335

Project Reports:

CM-03500 The Distribution of Drugs between Blood, Brain, and Cerebrospinal Fluid 345

CM-03516 Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents 352

Laboratory of Toxicology

Summary Report 357

Project Reports:

CM-03518 L-Asparagine: Synthesis, Biological Role and Hydrolysis Streptozotocin: Alkylation of Nucleic Acids 366

CM-06110 The Pathology of Drug-Induced Alterations in Animals and Man 375

CM-06122 Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs 382

CM-06123 Computer Methods for Quantification of Structure-Activity Relationships 390

Laboratory of Chemical Pharmacology

Summary Report 393

Project Reports:

CM-03502 Folic Acid Antagonists in Experimental Solid Tumors *In Vivo* 400

Page

CM-03505 Potential Embryotoxic, Carcinogenic and Other Adverse Effects of Anticancer Agents 403

CM-03506 Pharmacology and Disposition of Antitumor Drugs 407

CM-03509 Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates 411

CM-06108 Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents 416

CM-06120 Kinetic Profile on Drug Action *In Vivo* 419

CM-06121 Purification and Properties of Carboxypeptidase G_1 422

CM-06124 Biologic Markers Program for Clinical Cancer 424

CM-06125 Pharmacology of Maytansine, Dimethylbenzylrifampicin, and Other Ansa Macrolides 427

CM-06126 Purification and Properties of Cytidine Deaminase 429

CM-06127 Modified Degradation Products of Normal Monkey Liver and Hepatoma tRNA 431

CM-06128 Pharmacologic Aspects of Pentamidine 432

CM-06129 Studies on the Clinical Pharmacology of Folic Acid Antagonists 433

Laboratory of Molecular Pharmacology

Summary Report 437

Project Reports:

CM-06112 Effects of Chemotherapeutic Agents on DNA 440

CM-06130 Production and Repair of Macromolecular Errors and Damage 448

CM-06131 *In Vitro* Models for Chemotherapy in Human Cells 451

Laboratory of Tumor Cell Biology

Summary Report 455

Project Report:

CM-06117 Studies on Mechanisms Leading to Neoplastic Transformation; On Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and On the Development of Biochemical Markers of Neoplastic Cells. 459

Page

ASSOCIATE DIRECTOR FOR MEDICAL ONCOLOGY

Summary Report 473

Project Reports:

CM-03423 *In Vitro* Marrow Granulocyte Culture 481

CM-06119 Cytogenetic and Hematopoietic Stem Cell Studies 485

CM-06308 Biostatistical Information Systems Unit 488

CM-03024 Clinical Trials and Related Studies 494

CM-06309 Laboratory Studies in Cancer 509

CM-03403 Clinical Trials and Miscellaneous Clinical Investigations 516

CM-03404 Immunologic Aspects of Cancer 525

CM-03405 Hematology Investigations 528

CM-03420 Kinetics of Cellular Proliferation 531

CM-06500 Clinical Pharmacology--Mechanism of Action, Physiologic Disposition, Metabolism, and Toxicity of Anticancer Agents 534

CM-06700 Clinical Program in Breast Carcinoma 538

CM-06701 Factors Controlling Lymphocyte Reactivity *in vitro* 546

CM-06702 Hormone Dependence of Human Breast Cancer 548

CM-03422 Study and Treatment of Childhood Cancer 551

CM-06810 Chemoimmunotherapy 563

CM-06820 Blood Component Transfusion and Bone Marrow Transplantation 574

CM-06830 Studies on Molecular Mechanisms of Productive Infection and Carcinogenesis Associated with DNA and RNA Tumor Viruses 588

Infectious Complications of Malignancy: Prevention and Therapy

Page

ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH CENTER

Office of the Associate Director

Summary Report 601

Clinical Branch

Summary Report 603

Microbiology Section

CM-06027 Microbiological Services for Clinical and Research Studies of Cancer Patients 605

CM-06039 Computerized Microbiology Reporting and Data Retrieval Systems 607

CM-06219 Studies on Cancer Patient Microbial Flora: Acquisition, Colonization and Infection 609

CM-06223 Studies on *Pseudomonas aeruginosa* in a Cancer Research Center 611

CM-06254 Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques 614

CM-06938 Environmental Studies 616

Medical Oncology Section

CM-06217 Prevention of Infections in Patients with Cancer 618

CM-06230 Diagnostic Laparatomy and Splenectomy for Staging Hodgkin's Disease 620

CM-06259 Extension of the Computerized Problem-Oriented Medical Information System (PROMIS) into the Cancer Treatment Area 622

CM-06263 Protocol for Staging and Treatment of Lymphosarcoma Reticulum Cell Sarcoma 624

CM-06264 Evaluation of the Types and Courses of Infectious Diseases in Cancer Patients 626

CM-06266 Therapy of Infection in Patients with Cancer 629

CM-06275 Cell Transfusion Therapy 631

CM-06276 The Chemotherapy of Metastatic Testicular Carcinoma 635

Page

CM-06277 The Chemotherapy of Adult Acute Leukemia 637

CM-06280 Combination Chemotherapy for Stage IIIB and IV Hodgkin's Disease 643

CM-06293 A Teaching and Consultation Service in Medical Oncology 645

CM-06295 Census: A Remote Access Patient Information Storage and Retrieval System 647

CM-06299 Therapy of Patients with Metastatic Sarcoma 649

CM-06913 Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer 652

CM-06914 Intensive Supportive Care Alone Versus Intensive Supportive Care Plus Radiotherapy for Localized Inflammatory Lesions in Patients with Acute Leukemia 654

CM-06915 Staging Laparotomy and Combined Modality Therapy in Stage IA and IIA Lymphocyte Predominant, Nodular Sclerosing, and Mixed Cellularity Hodgkin's Disease 656

CM-06916 Pilot Protocol for the Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma 658

CM-06917 Cyclophosphamide, Adriamycin, Vincristine and Prednisone Versus Cyclophosphamide, Vincristine, and Prednisone for Disseminated Non-Hodgkin's Lymphoma 660

CM-06918 Streptozotocin, Bleomycin, CCNU, and Adriamycin for the Treatment of Advanced, Refractory Hodgkin's Disease 662

CM-06919 Initial Drug Therapy for Metastatic Renal Cell Carcinoma 664

CM-06920 5-Azacytidine Versus Guanazole for the Therapy of Previously Treated Adult Acute Non-Lymphocytic Leukemia 666

CM-06921 Adriamycin Therapy for Advanced Mycosis Fungoides 667

CM-06922 Epidemiology Investigations of 1)Prior Social Interaction Among Patients with Leukemia and Lymphoma and 2) the Possible Association between Environmental Solvents and Cancer Induction 669

Page

CM-06928 High Dose Methotrexate (HDMTX) for the Therapy of Advanced, Refractory Hodgkin's Disease, Non-Hodgkin's Lymphoma and Solid Tumors 671

CM-06929 A Pilot Study of Combination Therapy with 5-Azacytidine and methylGAG in Previous Treated Adults with Acute Nonlymphocytic Leukemia 673

Neurosurgery Service

CM-06024 Development of a Neurosurgical Service with Primary Interest in the Treatment of Brain Tumors at BCRC, NCI 675

CM-06204 Toxicity of Intracerebral Chemotherapy in Monkeys 678

CM-06282 The Brain Tumor Study Group 679

CM-06904 Studies on the Anti-Tumor Activity of methyl CCNU and Related Compounds at the Subcellular Level 682

CM-06906 Intravenous CCNU Phase I Study 684

CM-06907 Colony Stimulating Factor (CSF) and Colony Forming Capacity (CFC) from Patients with Malignant Neoplasms, and the Effects of Chemotherapy Upon CSF and CFC 686

CM-06908 Intravenous Methyl CCNU (NSC-95441) and PTG (NSC-122819) in the Treatment of Central Nervous System Neoplasms (Phase II) 688

CM-06909 Phase II Evaluation of Intrathecal N,N'N"-Triethylenethiophosphoramide (ThioTEPA-NSC-6396) in Meningeal Neoplasms 690

CM-06910 *In Vitro* Metabolism of CCNU and Methyl CCNU 692

CM-06911 *In Vivo* Metabolism of 3-Cyclohexyl Substituted in 1-Chloroethyl 1-Nitrosoureas 693

CM-06930 Alkylation of DNA in the Rat Glioma Model by Intravenous CCNU 695

CM-06931 Intravenous CCNU Phase II Colorectal Adenocarcinoma Study 697

Page

Clinical Research Pharmacy Service

CM-06043 Automated Monitoring of Drug Therapy 699

CM-06236 Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History 701

CM-06237 Development of a Clinical Research Pharmacy Service 703

CM-06238 Development of a Unit Dose Service and the Preparation of Parenteral Drugs 705

CM-06912 Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System 707

CM-06937 A Patient Oriented Outpatient Drug Distribution Service 709

Laboratory of Pharmacology

Summary Report 711

Biochemistry Section

CM-06012 Biochemical Pharmacology of Cancer Chemotherapeutic Agents 714

CM-06014 Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation 716

CM-06016 Pharmacodynamics of Cancer Chemotherapeutic Agents 718

CM-06241 Clinical Pharmacology of Cancer Chemotherapeutic Agents 721

Enzymology and Drug Metabolism Section

CM-06210 Development of Specific RNases as an Aid in Sequencing of tRNA from Murine Leukemia Viruses 723

CM-06242 Polyamines and Cancer, Functional Significance 725

CM-06247 Purification and Study of Bacterial and Mammalian Nucleases with Differing Residue Specificities 727

CM-06902 Characterization of Viral RNA Isolated from AKR Murine Leukemia Virus 729

CM-06903 Development of Specific DNases as an Aid in the Sequencing of DNA from Various Viral Tumors 731

		Page
CM-06925	Studies on the Metabolism, Function, and Storage of Human Blood Platelets	733
CM-06926	Role of Polyamines in Regulating Activity and Specificity of Various Nucleases	735
CM-06927	Properties of the *Enterobacter* Nuclease; Interaction of Nucleases with the Polyamines	737
CM-06933	Influence of Renal Insufficiency on Levels of Serum Ribonuclease in Patients with Multiple Myeloma	739
CM-06934	Poly A Inhibition of Ribonuclease Associated with an Endogenous Inhibitor: Effects of Spermidine and Ionic Strength	740
CM-06935	Isolation and Characterization of a Human Liver Ribonuclease	742
CM-06936	The Properties of Protein Inhibitors of Ribonuclease Activity	743

Immunology and Cell Biology Section

CM-06020	Mechanism of Human Lymphocyte Reactivity to Non-Self	744
CM-06249	Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients	746
CM-06274	Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody: The Characterization of Reactivity with the Complement System *in vivo* and Subsequent Effects	748
CM-06292	Immune Adjuvants	750
CM-06932	The Correlation of Abnormal Nuclease Activity to the Prediction and Presence of Cancer	752

Research Pathology Section

CM-06250	Ultrastructural and Tissue Culture Studies of Leukemic Cells of Patients before and During Treatment and of Normal Donor Granulocytes harvested for Transfusion Therapy	754

Page

CM-06272 Studies of Myelomonocytic Leukemia in an Animal Model System: Light and Electron Microscopy, Biological Experiments and Chemotherapy 756

CM-06923 Fine Structure and Cytochemistry of Human Neoplastic Solid Tumors 758

CM-06924 Ultrastructural Studies of Aberrant Cells in Possible Preleukemic States 759

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

Summary Report 761

Literature Research Section 805

Combined Modality Branch 807

Investigational Drug Branch 813

ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Vincent T. DeVita, Jr., M.D.
Director

The Division of Cancer Treatment has the responsibility for the development and coordination of cancer treatment within the National Cancer Program. The Division is divided into two major areas - clinical therapeutic research and basic research related to developmental therapeutics. The program has four major subdivisions on the campus at Bethesda and the Baltimore Cancer Research Center located at the University of Maryland in Baltimore, Maryland. Each is headed by an Associate Director.

The clinical program is divided into an extramural and intramural component. The intramural component is located on the campus in Bethesda, at the Veterans Hospital in Washington, D. C. and at the Baltimore Cancer Research Center Clinical Branch. These facilities encompass a total of 178 beds. In Bethesda the clinical area consists of the Medicine Branch, the Pediatric Oncology Branch and the VA Medical Oncology Branch, all formerly in the area designated Medical Oncology. With the recent transfer of the Radiation and Surgery Branches to DCT, this program, now designated the Clinical Program, will be headed by the Clinical Director.

The intramural program in its entirety has major programs in 13 different tumors as follows: Hodgkin's disease, non-Hodgkin's lymphoma, acute myelocytic leukemia, acute lymphocytic leukemia of childhood, carcinoma of the breast, carcinoma of the ovary, malignant melanoma, carcinoma of the pancreas, osteogenic sarcoma, carcinoma of the lung, head and neck cancers, Ewing's sarcoma, brain tumors, and more recently, carcinoma of the prostate. These intramural research programs are supplemented by collaborative clinical trials, under contract with the Division, in carcinoma of the breast, ovary, malignant melanoma, carcinoma of the pancreas, other sarcomas, lung cancer, testicular carcinomas, brain tumors, bladder cancer as well as contracts concerned with the development of new drug trials in a large variety of advanced solid tumors. Each of the Branches involved, in addition to conducting therapeutic trials, carries out major clinical research programs related to the natural history and biology of the specific cancers. These include pharmacokinetic studies during new drug trials, the study of the immunologic defects associated with cancer, the detection of tumor-associated antigens, the conduct and monitoring of immunotherapeutic trials, studies in drug metabolism, enzymology, viral oncology, studies on cell kinetics of both normal and tumor tissue, cytogenic alterations associated with malignant diseases and others.

A major program in supportive care is conducted at the Clinical Center. Histocompatibility typing for platelet and white cell transfusion support and the use of the protected environment as an adjunct to chemotherapeutic trials in leukemia, lymphomas and selected solid tumors are under study.

In addition, allogenic and autologous bone marrow transplantation programs are conducted in patients with refractory leukemia, lymphoma and aplastic anemia.

Where high volume, not available to the Clinical Center, is required or new leads are available for pursuit in cancers not normally seen at the Clinical Center, these programs are conducted in collaboration with other institutions utilizing the contract mechanism. The monitoring of the clinical trials both intramurally and extramurally is performed by the Cancer Therapy Evaluation Program which monitors the clinical contract program utilizing both its own staff and the expertise of each of the investigators within the Clinical Center with expertise in the 13 cancers mentioned. These investigators serve as a source of input and scientific base for the collaborative programs.

Major events in the past year have included: the utilization of a drug combination (CMF) developed in the Medicine Branch in an adjuvant program through one of the collaborative efforts with the Cancer Institute in Milan, Italy--this program appears to be the most effective adjuvant treatment of breast cancer; the demonstration that reticulum cell sarcoma (diffuse histiocytic lymphoma) is curable with a drug combination (10 year disease-free survival); the continued demonstration of the long disease-free survivals in Hodgkin's disease; and the identification of the nature of the cell of origin of childhood leukemia and the relationship of cell type to the prognosis of this disease.

The preclinical studies are in three areas in the Division: Drug Research and Development, Experimental Therapeutics and the laboratory program of the Baltimore Cancer Research Center. Drug Research and Development has as its primary task the search for and development of new antitumor agents. The identification of new compounds through screening in murine tumors, forms of primary method for recognition of antitumor properties of chemicals. This is done either by random screening or by screening compounds with known or suspected biologic activity. A major collaborative program exists concerned with the synthesis of anticancer agents and formulation for clinical trials. An intramural scientific program serves as the scientific base for the conduct of the collaborative program. Major accomplishments in the past year have been the development of a new improved synthetic procedure for the procurement of the highly active clinical compound, methotrexate. The new method is rapid and simple and provides this drug in high purity at low cost. In addition, a synthetic procedure has been developed, through the collaborative program, which provides the correct stereochemistry at the bridging carbons of the dimeric halves of the vinca-alkaloids. This step should lead to the development of analogues of vincristine, a highly active drug in the clinic, an approach which has not been possible in the past.

In support of the Drug Development program, the Experimental Therapeutics program conducts both an intramural research program and an extramural contract program concerned with the pharmacology, toxicology and mode of action of cancer chemotherapeutic agents. The Experimental Therapeutics program also conducts a major effort in molecular biology and biologic markers. These studies cover a broad range of drug-host interaction at the whole body, cellular, and molecular level. Through the toxicology program of Experimental Therapeutics, data is obtained to facilitate meeting FDA requirements for chemotherapeutic and immunotherapeutic agents for entry into clinical trials.

A major achievement in the laboratories of this program in the past year has been the isolation of a complete virus from the laboratory grown cells of a patient with myelogenous leukemia.

In summary, the programs of the Division span basic research at the molecular level and the actual treatment of thousands of patients with cancer. With the plethora of testable clinical tools for the treatment of patients with cancer, the program of the Division will emphasize their application in clinical trials to a greater degree in the coming year.

CODE FOR DIVISION OF CANCER TREATMENT PROGRAM LOGIC

Stage	Code	Description
I	1XX	Acquisition of new agents (includes studies of cellular control mechanisms).
IIA	2AX	Determination of anti-tumor activity of new agents.
IIB	2B1	Develop acceptable experimental formulation.
IIB	2B2	Detailed evaluation of new agents--dose, route, and schedule dependency (*in vivo* and *in vitro* systems). Cell kinetics.
IIB	2B4	Procurement of sufficient amounts of new agent for preclinical studies.
III	3X1	Toxicology and pharmacology in large animals (dogs and monkeys). Pharmacokinetics.
III	3X2	Production and formulation for clinical trials.
IV	4X1	Clinical Trials*-Phase I. Determination of tolerated dose. Cell kinetics.
IV	4X2	Clinical pharmacology--initial pharmacologic studies in man associated with early clinical trials. Pharmacokinetics.
V	5XX	Clinical Trials*--Phase II. Initial therapeutic trials. Development of "markers".
VI	6X1	Clinical Trials*--Phase III. Initial eradicative trials in man. Agent used singly or in combination with other agents in comparative study. Research, development and subsequent evaluation in supportive care (protected environment, platelet and leukocyte transfusions, antibiotics, etc.)
VI	6X2	Toxicologic and pharmacologic studies in large animals and man associated with further development of a drug. Microsomal enzyme. Molecular biology.
VII	7XX	Clinical Trials*--adjuvant studies (radiotherapy, surgery, immunotherapy). Determination of role of agent in total care. Rehabilitation.
---	8XX	Program management. Includes administration, dissemination of information to medical and scientific community. Generalized D.P.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

Linear Array Research:	The sequence of essential and minimum operational activities required to evaluate new agents, integrate them into a comprehensive therapy regimen and introduce them into medical practice.
Supplementary Array Research:	Research which, though not essential to operational success, contributes to methodological improvement of Linear Array activities.
Exploratory Array Research:	Research activities aimed at understanding relevant phenomena which, if successfully elucidated and exploited, could have an important impact upon cancer therapy.

TABLE I

Type of Activity By Stage of Program Logic		No. of Contracts	Annual Level[1]		(Percent)	
TOTAL		275[2]		63,225,824		
Stage I				10,004,289		15.82
1XX	Acquisition of New Agents	157	10,004,289		15.82	
Stage IIA				10,410,122		16.46
2AX	Determination of Anti-tumor Activity	132	10,410,122		16.46	
Stage IIB				10,674,136		16.88
2B1	Experimental Formulation Development	7	256,401		0.41	
2B2	Detailed Evaluation of New Agents	156	8,545,097		13.51	
2B4	Procure. of Agents for Preclinical Studies	16	1,872,638		2.96	
Stage III				7,318,319		11.58
3X1	Toxicology and Pharm. in Large Animals	54	4,770,599		7.55	
3X2	Product. and Formulation for Clinical Trials	21	2,547,720		4.03	
Stage IV				3,370,327		5.33
4X1	Clinical Trials - Phase I	19	2,435,838		3.85	
4X2	Initial Clinical Pharm. Studies in Man	11	934,489		1.48	
Stage V				4,198,966		6.64
5XX	Clinical Trials - Phase II	52	4,198,966		6.64	
Stage VI				9,832,557		15.56
6X1	Clinical Trials-Phase III	52	6,585,573		10.42	
6X2	Tox. & Pharm. Studies in Large Animals and Man	37	3,246,984		5.14	
Stage VII				5,612,806		8.88
7XX	Clinical Trials-Adjuvant Studies	38	5,612,806		8.88	
Program Mgt.				1,804,302		2.85
8XX	Administration and Information Functions	20	1,804,302		2.85	

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[1] FY'75 funding requirements as of March 31, 1975. Not included are funds utilized for direct purchase of clinical drugs (approximately $6,600,000). Also included are 11 Schedule A contracts ($4,338,709) and 14 Schedule B contracts ($8,750,692).

[2] Since many contracts involve more than one activity, the totals of Stages I through VII and the program management category do not correspond with the total number of contracts.

TABLE II

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS	$
AGRICULT.,DEPT.OF (YM40001)	350,000
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (NM12308)	76,931
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (NM33750)	120,200
ARS/SPRAGUE-DAWLEY (NM12007)	16,943
ARS/SPRAGUE-DAWLEY (NM53813)	91,224
ARS/SPRAGUE-DAWLEY (NM53846)	29,414
ARTHUR D. LITTLE, (NM33727)	105,745
ARTHUR D. LITTLE, (NM23711)	12,476
ASSOCIATED BIOMEDIC SYSTEMS, INC. (NM02271)	168,923
BATTELLE MEM.INST (NM02008)	7,848
BELLAIRE ACRES (NM40900)	1,210
BELLAIRE ACRES (NM40901)	5,470
BRISTOL MYERS CO. (NM43759)	191,878
BRITISH COLUMBIA, UNIV. OF (NM23223)	70,543
CHARLES RIVER BREEDING LABS. (NM40872)	1,334
CHARLES RIVER BREEDING LABS. (NM53812)	102,162
CHARLES RIVER BREEDING LABS. (NM50021)	20,140
CHARLES RIVER BREEDING LABS. (NM53848)	27,312
CHAS.RIV.BREEDING LABS. (NM22004)	37,910

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
CHEM. ABSTRACTS SERVICE (NM43722)	515,000
CHEM.ABSTR.SERVICE (NM51021)	49,600
CHESTER BEATTY RESEARCH INST. (NM43736)	16,500
COLLABORATIVE RES., RESEARCH, INC. (NM12326)	53,910
COLLABORATIVE RES., RESEARCH, INC. (NM60506)	89,544
COLLEGE OF MEDICINE & DENTISTRY OF NEW JERSEY (NM43760)	23,649
COLORADO, UNIV. OF (NM43779)	72,905
DOW CHEMICAL CO. (NM33724)	148,915
FLOW LABORATORIES, INC. (NM40894)	4,505
GEORGETOWN UNIV. (NM43732)	44,000
HARLAN INDUSTRIES (NM40932)	4,474
HARLAN INDUSTRIES (NM40931)	1,343
HARLAN INDUSTRIES (NM23218)	0
HAWAII, UNIV. OF (NM33747)	72,564
HEALTH RESEARCH, INC. (NM02035)	17,594
IIT RESEARCH INSTITUTE (NM43755)	42,530
IIT RESEARCH INSTITUTE (NM53829)	11,438
IIT RESEARCH INST. (NM81021)	1,620
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (NM22078)	103,905
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (NM23208)	55,000

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
INSTITUT JULES BORDET (NM53840)	17,100
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (NM33720)	24,940
JACKSON LABORATORY (NM50003)	12,995
JAPANESE FD. FOR CANCER RESEARCH (NM22054)	5,500
KANSAS, UNIVERSITY OF (NM53819)	13,745
LAB.SUPPLY CO. (NM0204[illegible])	1,957
LABORATORY SUPPLY COMPANY (NM40893)	1,286
LABORATORY SUPPLY COMPANY (NM40892)	4,343
LABORATORY SUPPLY CO. (NM53775)	11,993
LEO GOODWIN INST. FOR CANCER RESEARCH (NM53814)	60,026
LEO GOODWIN INST. FOR CANCER RESEARCH (NM33729)	1,320
LITTON BIONETICS, INC.,(NM12340)	512,613
LITTON BIONETICS, INC.,(NM22062)	46,406
LITTON BIONETICS, INC. (NM53778)	9,325
LITTON BIONETICS, INC. (N025423)	795,744
M.I.T. (NM43712)	60,137
MIAMI, UNIV. OF (NM02225)	2,417
MICRO. ASSOC., INC. (NM43793)	106,288
MICRO. ASSOC., INC. (NM43761)	374,345
MICRO. ASSOC.,INC. ASSOCIATES, INC. (NM33728)	223,247

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
MICROBIOLOGICAL ASSOCIATES, INC. (NM53818)	22,246
MURPHY BREEDING LABS (NM40876)	813
MURPHY BREEDING LABS (NM40875)	2,487
PARKE, DAVIS AND (NM43754)	478,102
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (NM33719)	146,600
PENNWALT CORP. (NM12309)	14,862
PFIZER, INC. (NM53805)	198,991
POMONA COLLEGE (NM33703)	41,700
RESEARCH TRIANGLE INST. (NM92019)	185,258
RHODE ISLAND, UNIV. OF (NM43778)	43,817
RHODE ISLAND, UNIV. OF (NM12312)	1,430
SCRIPPS CLINIC & RES. FD. (NM22042)	9,000
SIMONSEN LABORATORIES (NM53847)	30,821
SIMONSEN LABORATORIES (NM53817)	52,399
SIMONSEN LABORATORIES (NM40883)	5,459
SIMONSEN LABORATORIES (NM40884)	866
SMALL BUSINESS ADMINISTRATION (NM43719)	15,731
SOUTHERN ANIM.FARMS (NM22013)	3,869
SOUTHERN ANIMAL FARMS (NM40888)	7,318
SOUTHERN ANIMAL FARMS (NM40889)	1,666

CONTRACT	DOLLAR LEVEL
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
SOUTHERN ANIMAL FARMS (NM53845)	13,836
SOUTHERN RESEARCH INSTITUTE (NM43762)	624,333
SPARTAN RES. ANIM., INC. (NM30858)	66
STARKS, C. P., INC. (NM53745)	348,579
TACONIC FARMS (NM40864)	4,158
TEXAS INBRED MICE (NM43711)	5,958
TEXAS INBRED MICE (NM40927)	2,218
TEXAS INBRED MICE (NM53816)	65,528
TULANE UNIVERSITY (NM53815)	25,199
UPJOHN COMPANY (NM43753)	711,588
UTAH, UNIV. OF (NM43806)	175,672
VALUE ENGINEERING COMPANY (NM33706)	116,198
VIRGINIA, UNIV.OF (NM12099)	180,000
WARF INSTITUTE, INC. (NM43707)	102,692
WASH. STATE UNIV. UNIVERSITY (NM22059)	5,143
WISCONSIN, BOARD OF REGENTS OF THE UNIVERSITY OF (NM33733)	40,000
WISCONSIN, UNIV. OF (NM12275)	47,500
YALE UNIVERSITY SCHOOL OF MEDICINE (NM53824)	29,303
YALE UNIVERSITY (NM12339)	63,000
TOTAL	8,876,697

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY	
ARS/SPRAGUE-DAWLEY (NM12007)	74,290
ARS/SPRAGUE-DAWLEY (NM53813)	410,502
ARS/SPRAGUE-DAWLEY (NM53846)	132,357
ARTHUR D. LITTLE, INC. (NM53765)	349,599
ARTHUR D. LITTLE, (NM33727)	407,859
BATTELLE MEM.INST (NM02008)	35,314
BELLAIRE ACRES (NM40900)	5,443
BELLAIRE ACRES (NM40901)	24,607
CHARLES RIVER BREEDING LABS. (NM40872)	5,995
CHARLES RIVER BREEDING LABS. (NM53812)	459,726
CHARLES RIVER BREEDING LABS. (NM50021)	90,625
CHARLES RIVER BREEDING LABS. (NM53848)	122,900
CHAS.RIV.BREEDING LABS.(NM22004)	170,591
COLLEGE OF MEDICINE & DENTISTRY OF NEW JERSEY (NM43760)	106,415
COLORADO,UNIV.OF OF, MEDICAL CENTER (NM12186)	20,254
FLOW LABORATORIES, INC. (NM40894)	20,270
HARLAN INDUSTRIES (NM40932)	20,128
HARLAN INDUSTRIES (NM40931)	6,038
HARLAN INDUSTRIES (NM23218)	0

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
HEALTH RESEARCH, INC. (NM33726)	37,639
HEALTH RESEARCH, INC. (NM02035)	79,123
IIT RESEARCH INSTITUTE (NM43755)	170,359
IIT RESEARCH INSTITUTE (NM53829)	62,901
IIT RESEARCH INST. (NM81021)	8,893
INSTITUT JULES BORDET (NM53840)	14,250
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (NM33720)	140,760
JACKSON LABORATORY (NM50003)	86,632
JAPANESE FD. FOR CANCER RESEARCH (NM22054)	11,000
KANSAS, UNIVERSITY OF (NM53819)	61,850
LAB.SUPPLY CO. (NM02041)	8,848
LABORATORY SUPPLY COMPANY (NM40893)	5,783
LABORATORY SUPPLY COMPANY (NM40892)	19,561
LABORATORY SUPPLY CO. (NM53775)	56,687
LEO GOODWIN INST. FOR CANCER RESEARCH (NM53814)	270,111
LEO GOODWIN INST. FOR CANCER RESEARCH (NM33729)	5,934
LITTON BIONETICS, INC. (NM33741)	90,804
LITTON BIONETICS, INC. (NM53778)	41,957
MIAMI, UNIV. OF (NM02225)	118,408
MICHIGAN DEPARTMENT DEPT. OF PUBLIC HEALTH (NM33702)	54,166

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
MICRO. ASSOC., INC. (NM43793)	61,320
MICRO. ASSOC.,INC. ASSOCIATES, INC. (NM33728)	128,794
MICROBIOLOGICAL ASSOCIATES, INC. (NM53818)	100,107
MURPHY BREEDING LABS (NM40876)	3,657
MURPHY BREEDING LABS (NM40875)	11,183
PARKE, DAVIS AND (NM43754)	84,370
PENNSYLVANIA, UNIVERSITY OF (NM53830)	6,427
RESEARCH TRIANGLE INST. (NM92019)	9,750
SCRIPPS CLINIC & RES. FD. (NM22042)	3,000
SIMONSEN LABORATORIES (NM53847)	138,686
SIMONSEN LABORATORIES (NM53817)	235,792
SIMONSEN LABORATORIES (NM40883)	24,562
SIMONSEN LABORATORIES (NM40884)	3,895
SOUTHERN ANIM. FARMS (NM22013)	24,855
SOUTHERN ANIMAL FARMS (NM40888)	32,923
SOUTHERN ANIMAL FARMS (NM40889)	7,497
SOUTHERN ANIMAL FARMS (NM53845)	65,396
SPARTAN RES. ANIM., INC. (NM30858)	291
TACONIC FARMS (NM40864)	18,711
TEXAS INBRED MICE (NM43711)	26,806

CONTRACT	DOLLAR LEVEL
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
TEXAS INBRED MICE (NM40927)	9,979
TEXAS INBRED MICE (NM53816)	294,873
TULANE UNIVERSITY (NM53815)	113,330
UPJOHN COMPANY (NM43753)	212,228
UTAH, UNIV. OF (NM43806)	38,561
VALUE ENGINEERING COMPANY (NM33706)	497,988
WASH.STATE UNIV. UNIVERSITY (NM22059)	23,139
YALE UNIVERSITY (NM12339)	21,000
YALE UNIVERSITY SCHOOL OF MEDICINE (NM33711)	17,674
TOTAL	6,025,413

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIB: EXPERIMENTAL FORMULATION, DEVELOPMENT	
BEN VENUE LABORATORIES, INC. (NM53811)	84,257
BEN VENUE LABS.,INC.LABORATORIES, INC. (NM43701)	4,935
IOWA, UNIV. OF (NM43743)	25,083
KANSAS, UNIV. OF (NM23217)	95,000
PHILIPS ROXANE LABS.LABORATORIES, INC. (NM12139)	14,642
TENNESSEE,UNIV.OF (NM02084)	20,000
UPJOHN COMPANY (NM43753)	12,484
TOTAL	256,401

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS	
ALLEGHENY GENERAL HOSPITAL (NM43730)	84,000
ARS/SPRAGUE-DAWLEY (NM12007)	39,094
ARS/SPRAGUE-DAWLEY (NM53813)	149,865
ARS/SPRAGUE-DAWLEY (NM53846)	48,321
ARTHUR D. LITTLE, INC. (NM53765)	378,719
ARTHUR D. LITTLE, (NM33727)	377,648
ARTHUR D. LITTLE, (NM23711)	24,946
ATOMIC ENERGY COMMISSION (YM40102)	7,650
ATOMIC ENERGY COMM. (YM10056)	24,000
BATTELLE MEM.INST (NM02008)	12,892
BELLAIRE ACRES (NM40900)	1,987
BELLAIRE ACRES (NM40901)	8,983
BRIGHAM YOUNG UNIV. (NM43790)	75,296
CALIFORNIA, UNIV. OF (NM43791)	97,730
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, QUEENS (NM23703)	883,400
CHARLES RIVER BREEDING LABS. (NM40872)	2,199
CHARLES RIVER BREEDING LABS. (NM53812)	167,936
CHARLES RIVER BREEDING LABS. (NM50021)	33,085
CHARLES RIVER BREEDING LABS. (NM53848)	44,858

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
CHAS.RIV.BREEDING LABS.(NM22004)	62,279
CHESTER BEATTY RESEARCH INST. (NM43736)	13,500
COLLEGE OF MEDICINE & DENTISTRY OF NEW JERSEY (NM43760)	38,850
FLOW LABORATORIES, INC. (NM40894)	7,400
HARLAN INDUSTRIES (NM40932)	7,348
HARLAN INDUSTRIES (NM40931)	2,204
HARLAN INDUSTRIES (NM23218)	0
HARVARD UNIVERSITY (NM53825)	52,376
HEALTH RESEARCH, INC. (NM02035)	28,886
HEALTH RESEARCH, INC. (NM43785)	52,191
IIT RESEARCH INSTITUTE (NM53829)	40,028
IIT RESEARCH INST. (NM81021)	5,659
INSTITUTE OF CANCER RESEARCH (NM23717)	31,100
JACKSON LABORATORY (NM50003)	116,953
JAPANESE FD. FOR CANCER RESEARCH (NM22054)	11,000
KANSAS, UNIVERSITY OF (NM53819)	22,580
LAB.SUPPLY CO. (NM02041)	3,230
LABORATORY SUPPLY COMPANY (NM40893)	2,111
LABORATORY SUPPLY COMPANY (NM40892)	7,141
LABORATORY SUPPLY CO. (NM53775)	39,245

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
LEO GOODWIN INST. FOR CANCER RESEARCH (NM53814)	98,612
LEO GOODWIN INST. FOR CANCER RESEARCH (NM33729)	2,166
LITTON BIONETICS, INC. (NM33741)	211,875
LITTON BIONETICS, INC.,(NM22062)	46,404
LITTON BIONETICS, INC. (NM53778)	15,317
LITTON BIONETICS, INC. (N025423)	944,945
MADRID, UNIVERSITY OF (NM53792)	28,400
MICHIGAN CANCER FOUNDATION (NM53789)	55,089
MICRO. ASSOC., INC. (NM43793)	179,872
MICRO. ASSOC.,INC. ASSOCIATES, INC. (NM33728)	377,796
MICROBIOLOGICAL ASSOCIATES, INC. (NM53818)	36,547
MINNESOTA, UNIV. OF (NM43788)	67,017
MURPHY BREEDING LABS (NM40876)	1,335
MURPHY BREEDING LABS (NM40875)	4,092
NAVAL WEAPONS CENTER(YM50101)	85,000
NOTRE DAME,UNIV.OF (NM23718)	35,000
PENNSYLVANIA, UNIVERSITY OF (NM53830)	14,995
RADIOBIOLOGICAL INSTITUTE (NM53763)	57,023
SIMONSEN LABORATORIES (NM53847)	50,631
SIMONSEN LABORATORIES (NM53817)	86,083

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
SIMONSEN LABORATORIES (NM40883)	8,967
SIMONSEN LABORATORIES (NM40884)	1,422
SLOAN-KETTERING INSTITUTE FOR CANCERRESEARCH (NM53820)	74,884
SMALL BUSINESS ADMINISTRATION (NM43719)	141,572
SOUTHERN ANIM.FARMS (NM22013)	26,512
SOUTHERN ANIMAL FARMS (NM40888)	12,019
SOUTHERN ANIMAL FARMS (NM40889)	2,737
SOUTHERN ANIMAL FARMS (NM53845)	45,274
SOUTHERN RESEARCH INSTITUTE (NM43784)	372,473
SPARTAN RES. ANIM., INC. (NM30858)	106
TACONIC FARMS (NM40864)	6,831
TEXAS INBRED MICE (NM43711)	9,786
TEXAS INBRED MICE (NM40927)	3,643
TEXAS INBRED MICE (NM53816)	107,652
TEXAS, UNIV. OF OF, SYSTEM CANCER CTR/MDA (NM43786)	81,586
TULANE UNIVERSITY (NM53815)	41,396
TULANE UNIVERSITY (NM33701)	23,709
UPJOHN COMPANY (NM43753)	124,840
VALUE ENGINEERING COMPANY (NM33706)	215,794
WASH. UNIV. UNIVERSITY SCHOOL OFMEDICINE (NM43731)	106,085

CONTRACT	DOLLAR LEVEL
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
WASH.STATE UNIV. UNIVERSITY (NM22059)	8,447
YALE UNIVERSITY SCHOOL OF MEDICINE (NM53824)	29,302
YESHIVA UNIV. (NM43787)	82,900
TOTAL	6,922,616

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LEVEL
STAGE IIB: INITIAL ANIMAL PHARM. STUDIES		
TOTAL		0

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IIB: PROC. OF AGENTS FOR PRE-CLIN. STUDIES	
ABBOTT LABORATORIES (NM43704)	122,273
ALDRICH CHEMICAL CO.COMPANY, INC. (NM23706)	94,341
MERCK AND CO., INC. (NM22002)	301,549
MONSANTO RES.CORP. (NM23715)	175,996
MONSANTO RES.CORP. (NM33709)	34,629
PARKE,DAVIS & CO. (NM23708)	163,570
PHARM-ECO (NM23707)	68,550
STANFORD RES.INST. (NM12162)	167,992
STARKS ASSOC., INC. INC. (NM23203)	206,239
TOTAL	1,335,139

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS	
ARTHUR D. LITTLE, (NM33727)	619,343
ASSOCIATED BIOMEDIC SYSTEMS, INC. (NM02271)	42,230
BATTELLE MEMORIAL INSTITUTE (NM43746)	2,020,200
COLORADO,UNIV.OF OF, MEDICAL CENTER (NM12186)	47,258
DOW CHEMICAL CO. (NM23712)	69,259
HAZLETON LABS. LABORATORIES, INC. (NM43705)	31,500
HAZLETON RESEARCH ANIMALS, INC. (NM41150)	39,728
HEALTH RESEARCH, INC. (NM33726)	69,899
JOHNS HOPKINS UNIVERSITY (NM43718)	37,589
LABORATORY SUPPLY CO. (NM53775)	1,090
LITTON BIONETICS, INC.,(NM12340)	341,741
LITTON BIONETICS, INC.,(NM22062)	92,803
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH (NM23242)	30,000
MARSHALL RESEARCH ANIMALS, INC. (NM50010)	48,060
MICRO. ASSOC., INC. (NM43793)	61,320
MICRO. ASSOC.,INC. ASSOCIATES, INC. (NM33729)	128,793
SOUTHERN ANIMAL FARMS (NM53845)	1,257
SOUTHERN RES.INST. (NM02083)	60,724
SOUTHERN RESEARCH INSTITUTE (NM43780)	420,201

CONTRACT	DOLLAR LEVEL
STAGE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS (CONTINUED)	
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM61156)	11,500
TULANE UNIVERSITY (NM33701)	1,247
WISCONSIN,UNIV.OF (NM12275)	47,500
TOTAL	4,223,249

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE III: PRODUCT. & FORM. FOR CLINICAL TRIALS	
ALDRICH CHEMICAL CO.COMPANY, INC. (NM23706)	40,431
BEN. VENUE LABORATORIES, INC. (NM53811)	758,308
BEN VENUE LABS.,INC.LABORATORIES, INC. (NM43701)	44,410
IOWA, UNIV. OF (NM43743)	100,330
MERCK AND CO., INC. (NM22002)	100,516
MONSANTO RES.CORP. (NM23715)	43,938
MONSANTO RES.CORP. (NM33709)	311,656
PARKE,DAVIS & CO. (NM23708)	163,569
PHARM-ECO (NM23707)	102,825
PHILIPS ROXANE LABS.LABORATORIES, INC. (NM12139)	58,564
STANFORD RES.INST. (NM12162)	41,908
STARKS ASSOC., INC. INC. (NM23203)	137,492
TENNESSEE,UNIV.OF (NM02084)	50,000
VALUE ENGINEERING COMPANY (NM53821)	71,123
TOTAL	2,035,220

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IV: CLINICAL TRIALS - PHASE I	
ATOMIC ENERGY COMMISSION (YM40102)	68,850
CHILDREN'S HOSPITAL OF LOS ANGELES (NM43803)	145,200
GEORGETOWN UNIV. (NM12123)	60,669
HAZLETON LABS.,INC. (NM33705)	74,982
JAPANESE FD. FOR CANCER RESEARCH (NM22054)	16,500
KANSAS, UNIV. OF (NM43802)	153,000
MARYLAND, UNIVERSITYOF (NM43748)	810,000
MAYO FOUNDATION (NM53838)	165,715
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES (NM43804)	161,700
MOUNT SINAI SCHOOL OF MEDICINE (NM53837)	116,000
PUBLIC HEALTH SERV. SERVICE (YM40003)	54,792
SIDNEY FARBER CANCERCENTER (NM53839)	94,210
TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA (NM43801)	247,800
VETERANS ADMIN. (YM40004)	262,200
YALE UNIVERSITY SCHOOL OF MEDICINE (NM33711)	4,220
TOTAL	2,435,838

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE IV: INITIAL CLIN. PHARM. STUDIES IN MAN	
CHICAGO, UNIV. OF (NM33748)	28,000
JEFFERSON MEDICAL COLLEGE (NM02136)	23,229
MARYLAND, UNIVERSITYOF (NM43748)	540,000
PUBLIC HEALTH SERV. SERVICE (YM40003)	36,528
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM61156)	2,300
UPJOHN COMPANY (NM43753)	187,260
WALTER REED ARMY MED. CENTER (YM40005)	688
YALE UNIVERSITY SCHOOL OF MEDICINE (NM32711)	4,484
TOTAL	822,489

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE V: CLINICAL TRIALS - PHASE II	
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (NM33713)	123,000
ARTHUR D. LITTLE, (NM23711)	24,946
ATOMIC ENERGY COMM. (YM10060)	85,000
AUTOMATION INDUST., INDUSTRIES, INC. (NM33717)	26,054
BOWMAN GRAY SCHOOL OF MED. (NM80950)	4,725
CALIFORNIA, UNIV. OF(NM43706)	23,131
CONNECTICUT, UNIV. OF (NM12181)	11,340
CONTROL DATA CORPORATION (NM33739)	8,100
DUKE UNIVERSITY (NM02030)	20,848
EMORY UNIV. (NM43764)	9,200
GEORGETOWN UNIV. (NM12123)	60,667
GEORGETOWN UNIV. (NM33745)	33,422
HAZLETON LABS.,INC. (NM33708)	201,767
HAZLETON LABS.,INC. (NM33705)	74,980
HEALTH RESEARCH, INC. (NM43714)	16,507
HEALTH RESEARCH, INC. (NM53834)	107,217
INDIANA UNIV.FDN. (NM02028)	6,758
IST. NAZION PER LO LO STUDIO E LA CURA DEI TUMORI (NM33714)	39,250
IST. NAZION PER LO STUD. E LA CURA DEI TUMORI (NM43726)	10,000

CONTRACT	DOLLAR LEVEL
STAGE V: CLINICAL TRIALS - PHASE II (CONTINUED)	
KENTUCKY, UNIV. OF (NM71173)	8,198
LEEDS, UNIV. OF (NM43727)	18,500
MAKERERE UNIV. COLLEGE COUNCIL (NM71343)	100,673
MARYLAND, UNIVERSITYOF (NM43748)	810,000
MAYO FOUNDATION (NM53772)	121,534
MAYO FOUNDATION (NM02066)	100,000
MISSOURI, CURATORS OF THE UNIVERSITY OF (NM12323)	130,000
MOUNT SINAI SCHOOL OF MEDICINE (NM43703)	27,813
NATIONAL NAVAL MEDICAL CENTER (YM50100)	7,876
NEW YORK UNIV. (NM71174)	12,559
OHIO STATE UNIV. (NM71175)	11,485
PUBLIC HEALTH SERV. SERVICE (YM40003)	54,792
RES. FDN. OF STATE UNIVERSITY OF NEW NEW YORK (NM33715)	24,170
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33710)	142,264
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33737)	75,368
VERMONT, UNIV. OF (NM33716)	15,669
VETERANS ADMIN. (YM40004)	524,400
WALTER REED ARMY MED. CENTER (YM40005)	15,753
TOTAL	3,087,966

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VI: CLINICAL TRIALS - PHASE III	
ARTHUR D. LITTLE, (NM23711)	56,130
ATOMIC ENERGY COMM. (YM40101)	127,200
AUTOMATION INDUST., INDUSTRIES, INC. (NM33717)	65,133
BOWMAN GRAY SCHOOL OF MED. (NM80950)	11,024
CALIFORNIA, UNIV. OF(NM43706)	53,970
CALIFORNIA,UNIV.OF (NM02092)	384,000
CAPETOWN, UNIV. OF (NM33738)	11,856
CHILDREN'S HOSPITAL OF LOS ANGELES (NM53831)	399,980
COMMUNITY BLOOD & PLASMA SERVICE (NC42150)	18,556
CONNECTICUT, UNIV. OF (NM12181)	26,460
CONTROL DATA CORPORATION (NM33739)	18,900
DUKE UNIVERSITY (NM02030)	48,644
GEORGETOWN UNIV. (NM12123)	91,000
GEORGETOWN UNIV. (NM33745)	56,841
HAZLETON LABS.,INC. (NM33705)	112,470
HAZLETON LABS.,INC. LABORATORIES, INC. (NM23701)	24,000
HEALTH RESEARCH, INC. (NM33734)	72,237
HEALTH RESEARCH, INC. (NM43714)	16,507
HEALTH RESEARCH, INC. (NM53834)	107,217

CONTRACT	DOLLAR LEVEL
STAGE VI: CLINICAL TRIALS - PHASE III (CONTINUED)	
HEBREW UNIV. (NM12127)	105,000
INDIANA UNIV.FDN. (NM02028)	15,766
IST. NAZION PER LO LO STUDIO E LA CURA DEI TUMORI (NM33714)	19,625
KENTUCKY,UNIV.OF (NM71173)	19,128
MAKERERE UNIV. COLLEGE COUNCIL (NM71343)	151,008
MARYLAND, UNIVERSITYOF (NM43748)	810,000
MAYO FOUNDATION (NM53772)	121,533
MOUNT SINAI SCHOOL OF MEDICINE (NM43703)	27,812
NATIONAL NAVAL MEDICAL CENTER (YM50100)	6,902
NEW YORK UNIV. (NM71174)	29,303
OHIO STATE UNIV. (NM71175)	26,796
OHIO STATE UNIV. (NM33736)	38,156
PUBLIC HEALTH SERV. SERVICE (YM40003)	54,792
RES. FDN. OF STATE UNIVERSITY OF NEW NEW YORK (NM33715)	60,425
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33710)	60,969
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33737)	150,734
TEXAS, UNIVERSITY OF(NM53832)	550,000
VERMONT, UNIV. OF (NM33716)	15,658
VETERANS ADMIN. (YM40004)	1,311,000
WALTER REED ARMY MED.CENTER(YM40005)	9,931
TOTAL	5,296,573

ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN	
BRITISH COLUMBIA, UNIV. OF (NM23223)	17,635
COLORADO STATE UNIV. (NM12192)	60,283
GEORGE WASH. UNIV. (NM12206)	71,641
HAZLETON LABS.,INC. LABORATORIES, INC. (NM43702)	390,000
HAZLETON LABS.,INC. (NM33708)	201,754
JEFFERSON MEDICAL COLLEGE (NM02136)	2,531
KANSAS,UNIV.OF (NM12184)	99,930
LITTON BIONETICS, INC.,(NM12340)	854,354
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH (NM23242)	30,000
MARYLAND, UNIVERSITYOF (NM43748)	540,000
MICRO. ASSOC. INC.(NB92177)	94,520
NATIONAL NAVAL MEDICAL CENTER (YM50100)	358
OREGON STATE UNIV. (NM23201)	100,309
PUBLIC HEALTH SERV. SERVICE (YM4CC03)	36,528
SOUTHERN CALIF. UNIVERSITY OF (NM23241)	59,278
SOUTHERN RES.INST. (NM02083)	91,035
SOUTHERN RESEARCH INSTITUTE (NM43780)	74,152
STANFORD RES.INST. (NM23200)	93,923
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM61156)	9,200

CONTRACT	DOLLAR LEVEL
STAGE VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN (CONTINUED)	
WALTER REED ARMY MED. CENTER (YM40005)	694
WASHINGTON HOSPITAL CENTER (NM43710)	37,000
TOTAL	2,865,275

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VII: CLINICAL TRIALS - ADJUVANT STUDIES	
AUTOMATION INDUST., INDUSTRIES, INC. (NM33717)	39,080
CALIF., UNIV. OF (NM43798)	91,650
CAPETOWN, UNIV. OF (NM33738)	17,782
CHILDREN'S CANCER RESEARCH FOUNDATION,INC. (NM43781)	58,000
CHILDREN'S CANCER RESEARCH FOUNDATION,INC. (NM43799)	107,830
CLEVELAND CLIN. FD. (NM33704)	41,000
GEORGETOWN UNIV. (NM12123)	91,000
GEORGETOWN UNIV. (NM33745)	41,775
HAZLETON LABS.,INC. (NM33705)	112,470
HAZLETON LABS.,INC. LABORATORIES, INC. (NM23701)	96,000
HEALTH RESEARCH, INC. (NM33734)	108,355
HEALTH RESEARCH, INC. (NM43794)	76,596
HEALTH RESEARCH, INC. (NM43782)	57,200
IST. NAZION PER LO LO STUDIO E LA CURA DEI TUMORI (NM33714)	19,625
IST. NAZION PER LO STUD. E LA CURA DEI TUMORI (NM43726)	40,000
MARYLAND, UNIVERSITYOF (NM43748)	1,620,000
MAYO FOUNDATION (NM43783)	55,300
MAYO FOUNDATION (NM43796)	76,209
MIAMI, UNIV. OF (NM43800)	74,513

CONTRACT	DOLLAR LEVEL
STAGE VII: CLINICAL TRIALS - ADJUVANT STUDIES (CONTINUED)	
MOUNT SINAI SCHOOL OF MEDICINE (NM53841)	150,782
NATIONAL NAVAL MEDICAL CENTER (YM5010C)	2,864
OHIO STATE UNIV. (NM33736)	57,234
PUBLIC HEALTH SERV. SERVICE (YM40003)	109,594
RES. FDN. OF STATE UNIVERSITY OF NEW NEW YORK (NM33715)	94,595
SOUTHERN CAL., UNIV.UNIVERSITY OF (NM43795)	70,259
SOUTHERN CALIFORNIA,UNIVERSITY OF (NM53842)	143,500
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33737)	150,734
VETERANS ADMIN. (YM40004)	524,400
WALTER REED ARMY MED.CENTER(YM40005)	7,191
YALE UNIV. (NM43797)	75,098
YALE UNIVERSITY (NM53843)	102,200
TOTAL	4,292,806

ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVEL
STAGE VIII: UNASSIGNED-ADMIN. & INFORM. FUNCTION	
AMERICAN CANCER SOCIETY, INC. (NM53807)	50,000
ARTHUR D. LITTLE, (NM23711)	6,236
AUTOMATION INDUST., INDUSTRIES, INC. (NM33732)	36,269
FRANKLIN INSTITUTE RESEARCH LABS. (NM53771)	298,449
GEORGETOWN UNIV. (NM33745)	25,965
INSTITUT JULES BORDET (NM53840)	25,650
JAPANESE FD. FOR CANCER RESEARCH (NM22054)	11,000
LITTON BIONETICS, INC. (NO25423)	746,009
MARYLAND, UNIVERSITYOF (NM43748)	270,000
NATIONAL ACADEMY OF SCIENCES (ND52103)	12,950
NATIONAL ACADEMY OF SCIENCES (NM53850)	20,000
NATIONAL ACADEMY OF SCIENCES (NM53849)	22,000
PUBLIC HEALTH SERV. SERVICE (YM40003)	18,264
RES. FDN. OF STATE UNIVERSITY OF NEW NEW YORK (NM33715)	72,510
TOTAL	1,614,302

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY CONTRACT

FOR FISCAL YEAR 1975
AS OF MARCH 31, 1975

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
ABBOTT LABORATORIES (NM43704)				$ 122,273
2B4	PROC. OF PRECLINICAL MATERIALS	- 100% -		
.F	OTHER	100%	122,273	
AGRICULT.,DEPT.OF (YM40001)				350,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	350,000	
ALDRICH CHEMICAL CO.COMPANY, INC. (NM23706)				134,772
2B4	PROC. OF PRECLINICAL MATERIALS	- 70% -	94,341	
A	SYNTHETICS PROCUREMENT	70%	94,340	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 30% -	40,431	
A	SYNTHETICS PRODUCTION	30%	40,432	
ALLEGHENY GENERAL HOSPITAL (NM43730)				84,000
2B2	VERIFICATION SCREEN	- 100% -		
A	DETAILED DRUG EVALUATION IN VITRO	50%	42,000	
B	DETAILED DRUG EVALUATION IN VIVO	50%	42,000	
AMERICAN CANCER SOCIETY, INC. (NM53807)				50,000
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%	50,000	
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (NM[illegible]2308)				76,981
1XX	ACQUISITION OF MATERIALS	- 100% -		
D	MARINE/ARTHPOPODS PROCUREMENT	100%	76,981	
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (NM33713)				123,000
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
Q	MARKERS	50%	61,500	
R	DATA PROCESSING AND SUPPORT	50%	61,500	
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (NM33750)				120,200
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	79%	94,958	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	10%	12,020	
E	BIOASSAY OF NATURAL PRODUCTS	11%	13,222	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
ARS/SPRAGUE-DAWLEY (NM12007)				$ 130,317
1XX	ACQUISITION OF MATERIALS	- 13% -	16,943	
E	BIOASSAY OF NATURAL PRODUCTS	6%	7,819	
F	ANIMALS	7%	9,122	
2AX	BASIC SCREEN	- 57% -	74,280	
C	RELATED NEW ASSAY DEVELOPMENT	5%	6,516	
D	ANIMALS	52%	67,765	
2B2	VERIFICATION SCREEN	- 30% -	39,094	
D	RELATED NEW ASSAY DEVELOPMENT	5%	6,516	
E	ANIMALS	25%	32,579	
ARS/SPRAGUE-DAWLEY (NM53813)				651,591
1XX	ACQUISITION OF MATERIALS	- 14% -	91,224	
E	BIOASSAY OF NATURAL PRODUCTS	2%	13,032	
F	ANIMALS	12%	78,191	
2AX	BASIC SCREEN	- 63% -	410,502	
C	RELATED NEW ASSAY DEVELOPMENT	3%	19,548	
D	ANIMALS	60%	390,955	
2B2	VERIFICATION SCREEN	- 23% -	149,865	
E	ANIMALS	23%	149,866	
ARS/SPRAGUE-DAWLEY (NM53846)				210,092
1XX	ACQUISITION OF MATERIALS	- 14% -	29,414	
E	BIOASSAY OF NATURAL PRODUCTS	7%	14,706	
F	ANIMALS	7%	14,706	
2AX	BASIC SCREEN	- 63% -	132,357	
D	ANIMALS	53%	111,349	
F	OTHER	10%	21,009	
2B2	VERIFICATION SCREEN	- 23% -	48,321	
E	ANIMALS	20%	42,018	
G	OTHER	3%	6,303	
ARTHUR D. LITTLE, INC. (NM53765)				728,308
2AX	BASIC SCREEN	- 48% -	349,589	
B	PRIMARY SCREENING IN VIVO	30%	218,492	
C	RELATED NEW ASSAY DEVELOPMENT	10%	72,831	
F	OTHER	8%	58,265	
2B2	VERIFICATION SCREEN	- 52% -	378,719	
B	DETAILED DRUG EVALUATION IN VIVO	43%	313,172	
D	RELATED NEW ASSAY DEVELOPMENT	9%	65,548	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
ARTHUR D. LITTLE, (NM33727)				$ 1,510,595
1XX	ACQUISITION OF MATERIALS	- 7% -	105,745	
E	BIOASSAY OF NATURAL PRODUCTS	7%	105,742	
2AX	BASIC SCREEN	- 27% -	407,859	
A	PRIMARY SCREENING IN VITRO	1%	15,106	
B	PRIMARY SCREENING IN VIVO	23%	347,437	
C	RELATED NEW ASSAY DEVELOPMENT	2%	30,212	
D	ANIMALS	1%	15,106	
2B2	VERIFICATION SCREEN	- 25% -	377,648	
A	DETAILED DRUG EVALUATION IN VITRO	1%	15,106	
B	DETAILED DRUG EVALUATION IN VIVO	21%	317,225	
D	RELATED NEW ASSAY DEVELOPMENT	2%	30,212	
E	ANIMALS	1%	15,106	
3X1	PHARMACOLOGY/TOXICOLOGY	- 41% -	619,343	
C	ANIMALS	3%	45,318	
E	OTHER	6%	90,636	
F	PHARMACOLOGY	32%	483,390	
ARTHUR D. LITTLE, (NM23711)				124,734
1XX	ACQUISITION OF MATERIALS	- 10% -	12,476	
H	OTHER	10%	12,473	
2B2	VERIFICATION SCREEN	- 20% -	24,946	
C	MOLECULAR BIOLOGY PROGRAM	15%	18,710	
D	RELATED NEW ASSAY DEVELOPMENT	5%	6,237	
5XX	CLINICAL TRIALS - PHASE II	- 20% -	24,946	
S	OTHER	20%	24,947	
6X1	CLINICAL TRIALS - PHASE III	- 45% -	56,130	
L	LEUKEMIA	10%	12,473	
R	PROTECTED ENVIRONMENT	10%	12,473	
T	OTHER	25%	31,184	
8XX	PROGRAM MANAGEMENT	- 5% -	6,236	
A	ADMINISTRATION	5%	6,237	
ASSOCIATED BIOMEDIC SYSTEMS, INC. (NM02271)				211,153
1XX	ACQUISITION OF MATERIALS	- 80% -	168,923	
H	OTHER	80%	168,922	
3X1	PHARMACOLOGY/TOXICOLOGY	- 20% -	42,230	
F	PHARMACOLOGY	20%	42,231	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ATOMIC ENERGY COMMISSION (YM40102)			$ 76,500
2B2 VERIFICATION SCREEN	- 10% -	7,650	
A DETAILED DRUG EVALUATION IN VITRO	5%	3,825	
C MOLECULAR BIOLOGY PROGRAM	5%	3,825	
4X1 CLINICAL TRIALS - PHASE I	- 90% -	68,850	
A CELL KINETICS	90%	68,850	
ATOMIC ENERGY COMM. (YM10056)			24,000
2B2 VERIFICATION SCREEN	- 100% -		
A DETAILED DRUG EVALUATION IN VITRO	70%	16,800	
D RELATED NEW ASSAY DEVELOPMENT	30%	7,200	
ATOMIC ENERGY COMM. (YM40101)			127,200
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
P BLOOD PRODUCTS	100%	127,200	
ATOMIC ENERGY COMM. (YM10060)			85,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
Q MARKERS	90%	76,500	
R DATA PROCESSING AND SUPPORT	10%	8,500	
AUTOMATION INDUST., INDUSTRIES, INC. (NM33732)			36,269
8XX PROGRAM MANAGEMENT	- 100% -		
B DISSEMINATION OF INFORMATION	92%	33,367	
C DATA PROCESSING AND SUPPORT	8%	2,902	
AUTOMATION INDUST., INDUSTRIES, INC. (NM33717)			130,267
5XX CLINICAL TRIALS - PHASE II	- 20% -	26,054	
R DATA PROCESSING AND SUPPORT	20%	26,053	
6X1 CLINICAL TRIALS - PHASE III	- 50% -	65,133	
S DATA PROCESSING AND SUPPORT	50%	65,134	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	39,080	
P DATA PROCESSING AND SUPPORT	30%	39,080	
BATTELLE MEM.INST (NM02098)			56,054
1XX ACQUISITION OF MATERIALS	- 14% -	7,848	
E BIOASSAY OF NATURAL PRODUCTS	2%	1,121	
F ANIMALS	12%	6,726	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 63% -	35,314	
C	RELATED NEW ASSAY DEVELOPMENT	3%	1,682	
D	ANIMALS	60%	33,632	
2B2	VERIFICATION SCREEN	- 23% -	12,892	
D	RELATED NEW ASSAY DEVELOPMENT	3%	1,682	
E	ANIMALS	20%	11,211	
	BATTELLE MEMORIAL INSTITUTE (NM43746)			$ 2,020,200
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	70%	1,414,140	
B	SPECIAL TOXICITY STUDIES	14%	282,828	
C	ANIMALS	1%	20,202	
D	DATA PROCESSING AND SUPPORT	15%	303,030	
	BELLAIRE ACRES (NM40900)			8,640
1XX	ACQUISITION OF MATERIALS	- 14% -	1,210	
E	BIOASSAY OF NATURAL PRODUCTS	2%	173	
F	ANIMALS	12%	1,037	
2AX	BASIC SCREEN	- 63% -	5,443	
C	RELATED NEW ASSAY DEVELOPMENT	3%	259	
D	ANIMALS	60%	5,184	
2B2	VERIFICATION SCREEN	- 23% -	1,987	
D	RELATED NEW ASSAY DEVELOPMENT	3%	259	
E	ANIMALS	20%	1,728	
	BELLAIRE ACRES (NM40901)			39,060
1XX	ACQUISITION OF MATERIALS	- 14% -	5,470	
E	BIOASSAY OF NATURAL PRODUCTS	2%	781	
F	ANIMALS	12%	4,687	
2AX	BASIC SCREEN	- 63% -	24,607	
C	RELATED NEW ASSAY DEVELOPMENT	3%	1,172	
D	ANIMALS	60%	23,436	
2B2	VERIFICATION SCREEN	- 23% -	8,983	
D	RELATED NEW ASSAY DEVELOPMENT	3%	1,172	
E	ANIMALS	20%	7,812	
	BEN VENUE LABORATORIES, INC. (NM53811)			842,565
2B1	FORMULATION	- 10% -	84,257	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	10%	84,257	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 90% -	758,308	
F	FORMULATION	70%	589,796	
G	ANALYTICAL AND QUALITY CONTROL	20%	168,513	
	BEN VENUE LABS.,INC.LABORATORIES, INC. (NM43701)		$	49,345
2B1	FORMULATION	- 10% -	4,935	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	10%	4,935	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 90% -	44,410	
F	FORMULATION	70%	34,542	
G	ANALYTICAL AND QUALITY CONTROL	20%	9,869	
	BOWMAN GRAY SCHOOL OF MED. (NM80950)			15,749
5XX	CLINICAL TRIALS - PHASE II	- 30% -	4,725	
J	BRAIN	30%	4,725	
6X1	CLINICAL TRIALS - PHASE III	- 70% -	11,024	
J	BRAIN	70%	11,024	
	BRIGHAM YOUNG UNIV. (NM43790)			75,296
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	75,296	
	BRISTOL MYERS CO. (NM43759)			191,878
1XX	ACQUISITION OF MATERIALS	- 100% -		
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	100%	191,878	
	BRITISH COLUMBIA, UNIV. OF (NM23223)			88,178
1XX	ACQUISITION OF MATERIALS	- 80% -	70,543	
A	SYNTHETICS PROCUREMENT	80%	70,542	
6X2	TOXICOLOGY/PHARMACOLOGY	- 20% -	17,635	
D	OTHER	20%	17,636	
	CALIF., UNIV. OF (NM43798)			81,650
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E	PANCREAS	100%	81,650	
	CALIFORNIA, UNIV. OF(NM43791)			87,730
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	87,730	
	CALIFORNIA, UNIV. OF(NM43706)			77,101
5XX	CLINICAL TRIALS - PHASE II	- 30% -	23,131	
J	BRAIN	30%	23,130	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 70% -	53,970	
J	BRAIN	70%	53,971	
CALIFORNIA,UNIV.OF (NM02092)			$	384,000
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
Q	TRANSFUSION RESEARCH	100%	384,000	
CAPETOWN, UNIV. OF (NM33738)				29,638
6X1	CLINICAL TRIALS - PHASE III	- 40% -	11,856	
C	LUNG	40%	11,855	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	17,782	
C	LUNG	60%	17,783	
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, QUEENS (NM23703)				883,400
2B2	VERIFICATION SCREEN	- 100% -		
B	DETAILED DRUG EVALUATION IN VIVO	90%	795,060	
D	RELATED NEW ASSAY DEVELOPMENT	10%	88,340	
CHARLES RIVER BREEDING LABS. (NM40872)				9,517
1XX	ACQUISITION OF MATERIALS	- 14% -	1,334	
E	BIOASSAY OF NATURAL PRODUCTS	2%	190	
F	ANIMALS	12%	1,142	
2AX	BASIC SCREEN	- 63% -	5,995	
C	RELATED NEW ASSAY DEVELOPMENT	3%	286	
D	ANIMALS	60%	5,710	
2B2	VERIFICATION SCREEN	- 23% -	2,188	
D	RELATED NEW ASSAY DEVELOPMENT	3%	286	
E	ANIMALS	20%	1,903	
CHARLES RIVER BREEDING LABS. (NM53812)				729,724
1XX	ACQUISITION OF MATERIALS	- 14% -	102,162	
E	BIOASSAY OF NATURAL PRODUCTS	2%	14,594	
F	ANIMALS	12%	87,567	
2AX	BASIC SCREEN	- 63% -	459,726	
C	RELATED NEW ASSAY DEVELOPMENT	3%	21,892	
D	ANIMALS	60%	437,834	
2B2	VERIFICATION SCREEN	- 23% -	167,836	
E	ANIMALS	23%	167,837	
CHARLES RIVER BREEDING LABS. (NM50021)				143,850
1XX	ACQUISITION OF MATERIALS	- 14% -	20,140	
E	BIOASSAY OF NATURAL PRODUCTS	2%	2,877	
F	ANIMALS	12%	17,262	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 63% -	90,625	
C RELATED NEW ASSAY DEVELOPMENT	3%	4,316	
D ANIMALS	60%	86,310	
2B2 VERIFICATION SCREEN	- 23% -	33,085	
D RELATED NEW ASSAY DEVELOPMENT	3%	4,316	
E ANIMALS	20%	28,770	
CHARLES RIVER BREEDING LABS. (NM53848)			$ 195,080
1XX ACQUISITION OF MATERIALS	- 14% -	27,312	
E BIOASSAY OF NATURAL PRODUCTS	7%	13,656	
F ANIMALS	7%	13,656	
2AX BASIC SCREEN	- 63% -	122,900	
D ANIMALS	53%	103,392	
F OTHER	10%	19,508	
2B2 VERIFICATION SCREEN	- 23% -	44,868	
E ANIMALS	20%	39,016	
G OTHER	3%	5,852	
CHAS RIV.BREEDING LABS.(NM22004)			270,780
1XX ACQUISITION OF MATERIALS	- 14% -	37,910	
E BIOASSAY OF NATURAL PRODUCTS	2%	5,416	
F ANIMALS	12%	32,494	
2AX BASIC SCREEN	- 63% -	170,591	
C RELATED NEW ASSAY DEVELOPMENT	3%	8,123	
D ANIMALS	60%	162,469	
2B2 VERIFICATION SCREEN	- 23% -	62,279	
E ANIMALS	23%	62,279	
CHEM. ABSTRACTS SERVICE (NM43722)			515,000
1XX ACQUISITION OF MATERIALS	- 100% -		
G DATA PROCESSING AND SUPPORT	100%	515,000	
CHEM.ABSTR.SERVICE (NM51021)			49,600
1XX ACQUISITION OF MATERIALS	- 100% -		
G DATA PROCESSING AND SUPPORT	100%	49,600	
CHESTER BEATTY RESEARCH INST. (NM43736)			30,000
1XX ACQUISITION OF MATERIALS	- 55% -	16,500	
A SYNTHETICS PROCUREMENT	55%	16,500	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN	- 45% -	13,500	
B DETAILED DRUG EVALUATION IN VIVO	45%	13,500	
CHICAGO, UNIV. OF (NM33748)			$ 28,000
4X2 CLINICAL PHARMACOLOGY	- 100% -		
A CLIN. PHARMACOLOGY & PHARMACOKINETICS	100%	28,000	
CHILDREN'S CANCER RESEARCH FOUNDATION,INC. (NM43781)			58,000
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
P STOMACH	100%	58,000	
CHILDREN'S CANCER RESEARCH FOUNDATION,INC. (NM43799)			107,830
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E PANCREAS	100%	107,830	
CHILDREN'S HOSPITAL OF LOS ANGELES (NM43803)			145,200
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
B PHARMACOLOGY/TOXICOLOGY	100%	145,200	
CHILDREN'S HOSPITAL OF LOS ANGELES (NM53831)			399,980
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
R PROTECTED ENVIRONMENT	100%	399,980	
CLEVELAND CLIN. FD. (NM33704)			41,000
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
Q OTHER	100%	41,000	
COLLABORATIVE RES., RESEARCH, INC. (NM12326)			53,910
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	53,910	
COLLABORATIVE RES., RESEARCH, INC. (NM60506)			89,544
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	89,544	
COLLEGE OF MEDICINE & DENTISTRY OF NEW JERSEY (NM43760)			168,914
1XX ACQUISITION OF MATERIALS	- 14% -	23,649	
E BIOASSAY OF NATURAL PRODUCTS	7%	11,824	
F ANIMALS	7%	11,824	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 63% -	106,415	
D ANIMALS	53%	89,524	
P OTHER	10%	16,891	
2B2 VERIFICATION SCREEN	- 23% -	38,950	
E ANIMALS	20%	33,783	
G OTHER	3%	5,067	
COLORADO STATE UNIV. (NM12192)			$ 60,283
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	100%	60,283	
COLORADO, UNIV. OF (NM43779)			72,905
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	72,905	
COLORADO,UNIV.OF OF, MEDICAL CENTER (NM12186)			67,512
2AX BASIC SCREEN	- 30% -	20,254	
A PRIMARY SCREENING IN VITRO	30%	20,254	
3X1 PHARMACOLOGY/TOXICOLOGY	- 70% -	47,259	
P PHARMACOLOGY	70%	47,258	
COMMUNITY BLOOD & PLASMA SERVICE (NC42150)			18,556
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
Q TRANSFUSION RESEARCH	100%	19,556	
CONNECTICUT, UNIV. OF (NM12181)			37,800
5XX CLINICAL TRIALS - PHASE II	- 30% -	11,340	
J BRAIN	30%	11,340	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	26,460	
J BRAIN	70%	26,460	
CONTROL DATA CORPORATION (NM33739)			27,000
5XX CLINICAL TRIALS - PHASE II	- 30% -	8,100	
R DATA PROCESSING AND SUPPORT	30%	8,100	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	18,900	
S DATA PROCESSING AND SUPPORT	70%	18,900	
DOW CHEMICAL CO. (NM33724)			149,915
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	148,915	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
DOW CHEMICAL CO. (NM23712)		$	69,259
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
B SPECIAL TOXICITY STUDIES	100%	69,259	
DUKE UNIVERSITY (NM02030)			69,492
5XX CLINICAL TRIALS - PHASE II	- 30% -	20,848	
J BRAIN	30%	20,849	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	49,644	
J BRAIN	70%	48,644	
EMORY UNIV. (NM43764)			9,200
5XX CLINICAL TRIALS - PHASE II	- 100% -		
N OTHER DISEASES	100%	9,200	
FLOW LABORATORIES, INC. (NM40894)			32,175
1XX ACQUISITION OF MATERIALS	- 14% -	4,505	
E BIOASSAY OF NATURAL PRODUCTS	2%	644	
F ANIMALS	12%	3,861	
2AX BASIC SCREEN	- 63% -	20,270	
C RELATED NEW ASSAY DEVELOPMENT	3%	965	
D ANIMALS	60%	19,305	
2B2 VERIFICATION SCREEN	- 23% -	7,400	
D RELATED NEW ASSAY DEVELOPMENT	3%	965	
E ANIMALS	20%	6,435	
FRANKLIN INSTITUTE RESEARCH LABS. (NM53771)			298,449
8XX PROGRAM MANAGEMENT	- 100% -		
B DISSEMINATION OF INFORMATION	100%	298,449	
GEORGE WASH. UNIV. (NM12206)			71,641
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	95%	68,059	
C DATA PROCESSING AND SUPPORT	5%	3,582	
GEORGETOWN UNIV. (NM12123)			303,336
4X1 CLINICAL TRIALS - PHASE I	- 20% -	60,669	
B PHARMACOLOGY/TOXICOLOGY	20%	60,667	
5XX CLINICAL TRIALS - PHASE II	- 20% -	60,667	
G TESTICULAR	3%	9,100	
J BRAIN	4%	12,133	
K LYMPHOMAS	5%	15,167	
L LEUKEMIA	4%	12,133	
N OTHER DISEASES	4%	12,133	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 30% -	91,000	
G	TESTICULAR	4%	12,133	
J	BRAIN	4%	12,133	
K	LYMPHOMAS	8%	24,267	
L	LEUKEMIA	8%	24,267	
N	OTHER DISEASES	6%	18,200	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	91,000	
G	TESTICULAR	3%	9,100	
J	BRAIN	4%	12,133	
K	LYMPHOMAS	8%	24,267	
L	LEUKEMIA	8%	24,267	
N	OTHER DISEASES	7%	21,234	
GEORGETOWN UNIV. (NM33785)			$	167,103
5XX	CLINICAL TRIALS - PHASE II	- 20% -	33,422	
R	DATA PROCESSING AND SUPPORT	20%	33,421	
6X1	CLINICAL TRIALS - PHASE III	- 40% -	66,841	
S	DATA PROCESSING AND SUPPORT	40%	66,841	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 25% -	41,775	
P	DATA PROCESSING AND SUPPORT	25%	41,776	
8XX	PROGRAM MANAGEMENT	- 15% -	25,065	
A	ADMINISTRATION	5%	8,355	
B	DISSEMINATION OF INFORMATION	5%	8,355	
C	DATA PROCESSING AND SUPPORT	5%	8,355	
GEORGETOWN UNIV. (NM43732)				44,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	100%	44,000	
HARLAN INDUSTRIES (NM40932)				31,950
1XX	ACQUISITION OF MATERIALS	- 14% -	4,474	
E	BIOASSAY OF NATURAL PRODUCTS	2%	639	
F	ANIMALS	12%	3,834	
2AX	BASIC SCREEN	- 63% -	20,128	
C	RELATED NEW ASSAY DEVELOPMENT	3%	959	
D	ANIMALS	60%	19,170	
2B2	VERIFICATION SCREEN	- 23% -	7,348	
D	RELATED NEW ASSAY DEVELOPMENT	3%	959	
E	ANIMALS	20%	6,390	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
HARLAN INDUSTRIES (NM40931)			$ 9,585
1XX ACQUISITION OF MATERIALS	- 14% -	1,343	
E BIOASSAY OF NATURAL PRODUCTS	2%	192	
F ANIMALS	12%	1,150	
2AX BASIC SCREEN	- 63% -	6,038	
C RELATED NEW ASSAY DEVELOPMENT	3%	288	
D ANIMALS	60%	5,751	
2B2 VERIFICATION SCREEN	- 23% -	2,204	
D RELATED NEW ASSAY DEVELOPMENT	3%	288	
E ANIMALS	20%	1,917	
HARLAN INDUSTRIES (NM23218)			0
1XX ACQUISITION OF MATERIALS	- 7% -		
F ANIMALS	7%		
2AX BASIC SCREEN	- 45% -		
C RELATED NEW ASSAY DEVELOPMENT	3%		
D ANIMALS	42%		
2B2 VERIFICATION SCREEN	- 48% -		
D RELATED NEW ASSAY DEVELOPMENT	5%		
E ANIMALS	43%		
HARVARD UNIVERSITY (NM53825)			52,376
2B2 VERIFICATION SCREEN	- 100% -		
C MOLECULAR BIOLOGY PROGRAM	100%	52,376	
HAWAII, UNIV. OF (NM33747)			72,564
1XX ACQUISITION OF MATERIALS	- 100% -		
B PLANT PRODUCTS PROCUREMENT	100%	72,564	
HAZLETON LABS. LABORATORIES, INC. (NM43705)			31,500
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	50%	15,750	
B SPECIAL TOXICITY STUDIES	50%	15,750	
HAZLETON LABS.,INC. LABORATORIES, INC. (NM43702)			390,000
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	100%	390,000	
HAZLETON LABS.,INC. (NM33708)			403,531
5XX CLINICAL TRIALS - PHASE II	- 50% -	201,767	
Q MARKERS	50%	201,766	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X2 TOXICOLOGY/PHARMACOLOGY	- 50% -	201,764	
A ANIMAL STUDIES	50%	201,766	
HAZLETON LABS.,INC. (NM33705)			$ 374,902
4X1 CLINICAL TRIALS - PHASE I	- 20% -	74,982	
B PHARMACOLOGY/TOXICOLOGY	20%	74,980	
5XX CLINICAL TRIALS - PHASE II	- 20% -	74,980	
G TESTICULAR	3%	11,247	
J BRAIN	4%	14,996	
K LYMPHOMAS	5%	18,745	
L LEUKEMIA	4%	14,996	
N OTHER DISEASES	4%	14,996	
6X1 CLINICAL TRIALS - PHASE III	- 30% -	112,470	
G TESTICULAR	4%	14,996	
J BRAIN	4%	14,996	
K LYMPHOMAS	8%	29,992	
L LEUKEMIA	8%	29,992	
N OTHER DISEASES	6%	22,494	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	112,470	
G TESTICULAR	3%	11,247	
J BRAIN	4%	14,996	
K LYMPHOMAS	8%	29,992	
L LEUKEMIA	8%	29,992	
N OTHER DISEASES	7%	26,243	
HAZLETON LABS.,INC. LABORATORIES, INC. (NM23701)			120,000
6X1 CLINICAL TRIALS - PHASE III	- 20% -	24,000	
Q TRANSFUSION RESEARCH	20%	24,000	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 80% -	96,000	
L LEUKEMIA	80%	96,000	
HAZLETON RESEARCH ANIMALS, INC. (NM41150)			39,728
3X1 PHARMACOLOGY/TOXICOLOGY	- 100% -		
A PROTOCOL TOXICITY STUDIES	50%	19,864	
B SPECIAL TOXICITY STUDIES	50%	19,864	
HEALTH RESEARCH, INC. (NM33734)			180,592
6X1 CLINICAL TRIALS - PHASE III	- 40% -	72,237	
C LUNG	40%	72,237	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	108,355	
C	LUNG	60%	108,355	
HEALTH	RESEARCH, INC. (NM33726)		$	107,538
2AX	BASIC SCREEN	- 35% -	37,639	
A	PRIMARY SCREENING IN VITRO	10%	10,754	
B	PRIMARY SCREENING IN VIVO	25%	26,885	
3X1	PHARMACOLOGY/TOXICOLOGY	- 65% -	69,899	
F	PHARMACOLOGY	65%	69,900	
HEALTH	RESEARCH, INC. (NM02035)			125,593
1XX	ACQUISITION OF MATERIALS	- 14% -	17,584	
F	ANIMALS	14%	17,583	
2AX	BASIC SCREEN	- 63% -	79,123	
D	ANIMALS	57%	71,588	
F	OTHER	6%	7,536	
2B2	VERIFICATION SCREEN	- 23% -	28,886	
E	ANIMALS	20%	25,119	
G	OTHER	3%	3,768	
HEALTH	RESEARCH, INC. (NM43714)			33,014
5XX	CLINICAL TRIALS - PHASE II	- 50% -	16,507	
G	TESTICULAR	50%	16,507	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	16,507	
G	TESTICULAR	50%	16,507	
HEALTH	RESEARCH, INC. (NM43794)			76,586
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E	PANCREAS	100%	76,586	
HEALTH	RESEARCH, INC. (NM43785)			52,191
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	52,191	
HEALTH	RESEARCH, INC. (NM43782)			57,200
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
F	STOMACH	100%	57,200	
HEALTH	RESEARCH, INC. (NM53834)			214,434
5XX	CLINICAL TRIALS - PHASE II	- 50% -	107,217	
A	BREAST	50%	107,217	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 50% -	107,217	
A	BREAST	50%	107,217	
HEBREW UNIV. (NM12127)			$	105,000
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
A	BREAST	25%	26,250	
B	COLON	75%	78,750	
IIT RESEARCH INSTITUTE (NM43755)				212,949
1XX	ACQUISITION OF MATERIALS	- 20% -	42,590	
E	BIOASSAY OF NATURAL PRODUCTS	15%	31,942	
G	DATA PROCESSING AND SUPPORT	5%	10,647	
2AX	BASIC SCREEN	- 80% -	170,359	
A	PRIMARY SCREENING IN VITRO	5%	10,647	
B	PRIMARY SCREENING IN VIVO	55%	117,122	
E	DATA PROCESSING AND SUPPORT	20%	42,590	
IIT RESEARCH INSTITUTE (NM53829)				114,367
1XX	ACQUISITION OF MATERIALS	- 10% -	11,439	
E	BIOASSAY OF NATURAL PRODUCTS	10%	11,437	
2AX	BASIC SCREEN	- 55% -	62,901	
B	PRIMARY SCREENING IN VIVO	41%	46,890	
C	RELATED NEW ASSAY DEVELOPMENT	4%	4,575	
D	ANIMALS	10%	11,437	
2B2	VERIFICATION SCREEN	- 35% -	40,028	
B	DETAILED DRUG EVALUATION IN VIVO	29%	33,166	
E	ANIMALS	6%	6,862	
IIT RESEARCH INST. (NM81021)				16,172
1XX	ACQUISITION OF MATERIALS	- 10% -	1,620	
E	BIOASSAY OF NATURAL PRODUCTS	10%	1,617	
2AX	BASIC SCREEN	- 55% -	8,893	
B	PRIMARY SCREENING IN VIVO	41%	6,631	
C	RELATED NEW ASSAY DEVELOPMENT	4%	647	
D	ANIMALS	10%	1,617	
2B2	VERIFICATION SCREEN	- 35% -	5,659	
B	DETAILED DRUG EVALUATION IN VIVO	29%	4,690	
E	ANIMALS	6%	970	
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (NM22078)				103,805
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	90%	93,425	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	10%	10,381	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (NM23008)				$ 55,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	50%	27,500	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	50%	27,500	
INDIANA UNIV.FDN. (NM02028)				22,524
5XX	CLINICAL TRIALS - PHASE II	- 30% -	6,758	
J	BRAIN	30%	6,757	
6X1	CLINICAL TRIALS - PHASE III	- 70% -	15,766	
J	BRAIN	70%	15,767	
INSTITUT JULES BORDET (NM53840)				57,000
1XX	ACQUISITION OF MATERIALS	- 30% -	17,100	
A	SYNTHETICS PROCUREMENT	25%	14,250	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	5%	2,850	
2AX	BASIC SCREEN	- 25% -	14,250	
A	PRIMARY SCREENING IN VITRO	5%	2,850	
B	PRIMARY SCREENING IN VIVO	20%	11,400	
8XX	PROGRAM MANAGEMENT	- 45% -	25,650	
B	DISSEMINATION OF INFORMATION	45%	25,650	
INSTITUTE OF CANCER RESEARCH (NM23717)				31,100
2B2	VERIFICATION SCREEN	- 100% -		
A	DETAILED DRUG EVALUATION IN VITRO	50%	15,550	
B	DETAILED DRUG EVALUATION IN VIVO	50%	15,550	
IOWA, UNIV. OF (NM43743)				125,413
2B1	FORMULATION	- 20% -	25,083	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	20%	25,083	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 80% -	100,330	
F	FORMULATION	70%	87,789	
G	ANALYTICAL AND QUALITY CONTROL	10%	12,541	
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (NM33720)				165,600
1XX	ACQUISITION OF MATERIALS	- 15% -	24,840	
A	SYNTHETICS PROCUREMENT	15%	24,840	
2AX	BASIC SCREEN	- 85% -	140,760	
B	PRIMARY SCREENING IN VIVO	85%	140,760	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
IST NAZION PER LO LO STUDIO E LA CURA DEI TUMORI (NM33714)			$ 78,500
5XX CLINICAL TRIALS - PHASE II	- 50% -	39,250	
A BREAST	25%	19,625	
B COLON	12%	9,420	
F STOMACH	13%	10,205	
6X1 CLINICAL TRIALS - PHASE III	- 25% -	19,625	
A BREAST	20%	15,700	
J BRAIN	5%	3,925	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 25% -	19,625	
A BREAST	20%	15,700	
J BRAIN	5%	3,925	
IST. NAZION PER LO STUD. E LA CURA DEI TUMORI (NM43726)			50,000
5XX CLINICAL TRIALS - PHASE II	- 20% -	10,000	
N OTHER DISEASES	20%	10,000	
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 80% -	40,000	
N OTHER DISEASES	80%	40,000	
JACKSON LABORATORY (NM50003)			216,580
1XX ACQUISITION OF MATERIALS	- 6% -	12,995	
E BIOASSAY OF NATURAL PRODUCTS	1%	2,166	
F ANIMALS	5%	10,829	
2AX BASIC SCREEN	- 40% -	86,632	
C RELATED NEW ASSAY DEVELOPMENT	3%	6,497	
D ANIMALS	37%	80,135	
2B2 VERIFICATION SCREEN	- 54% -	116,953	
C MOLECULAR BIOLOGY PROGRAM	5%	10,829	
D RELATED NEW ASSAY DEVELOPMENT	49%	106,124	
JAPANESE FD. FOR CANCER RESEARCH (NM22054)			55,000
1XX ACQUISITION OF MATERIALS	- 10% -	5,500	
A SYNTHETICS PROCUREMENT	5%	2,750	
C FERMENTATION/ANTIBIOTICS PROCUREMENT	5%	2,750	
2AX BASIC SCREEN	- 20% -	11,000	
A PRIMARY SCREENING IN VITRO	2%	1,100	
B PRIMARY SCREENING IN VIVO	18%	9,900	
2B2 VERIFICATION SCREEN	- 20% -	11,000	
A DETAILED DRUG EVALUATION IN VITRO	2%	1,100	
B DETAILED DRUG EVALUATION IN VIVO	18%	9,900	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
4X1	CLINICAL TRIALS - PHASE I	- 30% -	16,500	
B	PHARMACOLOGY/TOXICOLOGY	30%	16,500	
8XX	PROGRAM MANAGEMENT	- 20% -	11,000	
B	DISSEMINATION OF INFORMATION	20%	11,000	
JEFFERSON MEDICAL COLLEGE (NM02136)			$	25,310
4X2	CLINICAL PHARMACOLOGY	- 90% -	23,229	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	90%	23,229	
6X2	TOXICOLOGY/PHARMACOLOGY	- 10% -	2,581	
A	ANIMAL STUDIES	10%	2,581	
JOHNS HOPKINS UNIVERSITY (NM43718)				37,589
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
E	OTHER	50%	18,795	
F	PHARMACOLOGY	50%	18,795	
KANSAS, UNIV. OF (NM43802)				153,000
4X1	CLINICAL TRIALS - PHASE I	- 100% -		
B	PHARMACOLOGY/TOXICOLOGY	100%	153,000	
KANSAS, UNIV. OF (NM23217)				95,000
2B1	FORMULATION	- 100% -		
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	100%	95,000	
KANSAS, UNIVERSITY OF (NM53819)				98,175
1XX	ACQUISITION OF MATERIALS	- 14% -	13,745	
E	BIOASSAY OF NATURAL PRODUCTS	2%	1,964	
F	ANIMALS	12%	11,781	
2AX	BASIC SCREEN	- 63% -	61,850	
C	RELATED NEW ASSAY DEVELOPMENT	3%	2,945	
D	ANIMALS	60%	58,905	
2B2	VERIFICATION SCREEN	- 23% -	22,580	
E	ANIMALS	23%	22,580	
KANSAS,UNIV.OF (NM12184)				99,980
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
B	HUMAN STUDIES	75%	74,985	
C	DATA PROCESSING AND SUPPORT	25%	24,995	
KENTUCKY,UNIV.OF (NM71173)				27,326
5XX	CLINICAL TRIALS - PHASE II	- 30% -	8,198	
J	BRAIN	30%	8,198	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1 CLINICAL TRIALS - PHASE III	- 70% -	19,128	
J BRAIN	70%	19,128	
LAB.SUPPLY CO. (NM02041)		$	14,045
1XX ACQUISITION OF MATERIALS	- 14% -	1,967	
E BIOASSAY OF NATURAL PRODUCTS	2%	281	
F ANIMALS	12%	1,685	
2AX BASIC SCREEN	- 63% -	8,848	
C RELATED NEW ASSAY DEVELOPMENT	3%	421	
D ANIMALS	60%	8,427	
2B2 VERIFICATION SCREEN	- 23% -	3,230	
D RELATED NEW ASSAY DEVELOPMENT	3%	421	
E ANIMALS	20%	2,809	
LABORATORY SUPPLY COMPANY (NM40893)			9,180
1XX ACQUISITION OF MATERIALS	- 14% -	1,286	
E BIOASSAY OF NATURAL PRODUCTS	2%	184	
F ANIMALS	12%	1,102	
2AX BASIC SCREEN	- 63% -	5,783	
C RELATED NEW ASSAY DEVELOPMENT	3%	275	
D ANIMALS	60%	5,508	
2B2 VERIFICATION SCREEN	- 23% -	2,111	
D RELATED NEW ASSAY DEVELOPMENT	3%	275	
E ANIMALS	20%	1,836	
LABORATORY SUPPLY COMPANY (NM40892)			31,050
1XX ACQUISITION OF MATERIALS	- 14% -	4,348	
E BIOASSAY OF NATURAL PRODUCTS	2%	621	
F ANIMALS	12%	3,726	
2AX BASIC SCREEN	- 63% -	19,561	
C RELATED NEW ASSAY DEVELOPMENT	3%	932	
D ANIMALS	60%	18,630	
2B2 VERIFICATION SCREEN	- 23% -	7,141	
D RELATED NEW ASSAY DEVELOPMENT	3%	932	
E ANIMALS	20%	6,210	
LABORATORY SUPPLY CO. (NM53775)			109,015
1XX ACQUISITION OF MATERIALS	- 11% -	11,993	
E BIOASSAY OF NATURAL PRODUCTS	2%	2,180	
F ANIMALS	9%	9,811	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 52% -	56,687	
C	RELATED NEW ASSAY DEVELOPMENT	3%	3,270	
D	ANIMALS	49%	53,417	
2B2	VERIFICATION SCREEN	- 36% -	39,245	
D	RELATED NEW ASSAY DEVELOPMENT	5%	5,451	
E	ANIMALS	31%	33,795	
3X1	PHARMACOLOGY/TOXICOLOGY	- 1% -	1,090	
C	ANIMALS	1%	1,090	
	LEEDS, UNIV. OF (NM43727)			$ 18,500
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
B	COLON	60%	11,100	
F	STOMACH	40%	7,400	
	LEO GOODWIN INST. FOR CANCER RESEARCH (NM53814)			429,749
1XX	ACQUISITION OF MATERIALS	- 14% -	60,026	
E	BIOASSAY OF NATURAL PRODUCTS	2%	8,575	
F	ANIMALS	12%	51,450	
2AX	BASIC SCREEN	- 63% -	270,111	
C	RELATED NEW ASSAY DEVELOPMENT	3%	12,862	
D	ANIMALS	60%	257,249	
2B2	VERIFICATION SCREEN	- 23% -	98,612	
E	ANIMALS	23%	98,612	
	LEO GOODWIN INST. FOR CANCER RESEARCH (NM33729)			9,420
1XX	ACQUISITION OF MATERIALS	- 14% -	1,320	
E	BIOASSAY OF NATURAL PRODUCTS	2%	188	
F	ANIMALS	12%	1,130	
2AX	BASIC SCREEN	- 63% -	5,934	
C	RELATED NEW ASSAY DEVELOPMENT	3%	283	
D	ANIMALS	60%	5,652	
2B2	VERIFICATION SCREEN	- 23% -	2,166	
E	ANIMALS	23%	2,167	
	LITTON BIONETICS, INC. (NM33741)			302,690
2AX	BASIC SCREEN	- 30% -	90,804	
C	RELATED NEW ASSAY DEVELOPMENT	30%	90,804	
2B2	VERIFICATION SCREEN	- 70% -	211,876	
A	DETAILED DRUG EVALUATION IN VITRO	70%	211,876	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
LITTON BIONETICS, INC., (NM12340)				$ 1,708,708
1XX	ACQUISITION OF MATERIALS	- 30% -	512,613	
H	OTHER	30%	512,612	
3X1	PHARMACOLOGY/TOXICOLOGY	- 20% -	341,741	
F	PHARMACOLOGY	20%	341,742	
6X2	TOXICOLOGY/PHARMACOLOGY	- 50% -	854,354	
B	HUMAN STUDIES	50%	854,354	
LITTON BIONETICS, INC., (NM22062)				185,619
1XX	ACQUISITION OF MATERIALS	- 25% -	46,406	
H	OTHER	25%	46,405	
2B2	VERIFICATION SCREEN	- 25% -	46,404	
C	MOLECULAR BIOLOGY PROGRAM	25%	46,405	
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	92,809	
F	PHARMACOLOGY	50%	92,810	
LITTON BIONETICS, INC. (NM53778)				66,599
1XX	ACQUISITION OF MATERIALS	- 14% -	9,325	
E	BIOASSAY OF NATURAL PRODUCTS	7%	4,662	
F	ANIMALS	7%	4,662	
2AX	BASIC SCREEN	- 63% -	41,957	
D	ANIMALS	53%	35,297	
F	OTHER	10%	6,660	
2B2	VERIFICATION SCREEN	- 23% -	15,317	
E	ANIMALS	20%	13,320	
G	OTHER	3%	1,998	
LITTON BIONETICS, INC. (NO25423)				2,486,698
1XX	ACQUISITION OF MATERIALS	- 32% -	795,744	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	31%	770,876	
G	DATA PROCESSING AND SUPPORT	1%	24,867	
2B2	VERIFICATION SCREEN	- 38% -	944,945	
E	ANIMALS	38%	944,945	
8XX	PROGRAM MANAGEMENT	- 30% -	746,009	
A	ADMINISTRATION	30%	746,009	
M.I.T. (NM43712)				60,137
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	60,137	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
MADRID, UNIVERSITY OF (NM53792)				$ 28,400
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	28,400	
MAKERERE UNIV. COLLEGE COUNCIL (NM71343)				251,681
5XX	CLINICAL TRIALS - PHASE II	- 40% -	100,673	
D	PEDIATRIC TUMORS	10%	25,168	
K	LYMPHOMAS	20%	50,336	
N	OTHER DISEASES	10%	25,168	
6X1	CLINICAL TRIALS - PHASE III	- 60% -	151,008	
D	PEDIATRIC TUMORS	20%	50,336	
K	LYMPHOMAS	20%	50,336	
N	OTHER DISEASES	20%	50,336	
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH (NM23242)				60,000
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	30,000	
F	PHARMACOLOGY	50%	30,000	
6X2	TOXICOLOGY/PHARMACOLOGY	- 50% -	30,000	
A	ANIMAL STUDIES	50%	30,000	
MARSHALL RESEARCH ANIMALS, INC. (NM50010)				48,060
3X1	PHARMACOLOGY/TOXICOLOGY	- 100% -		
A	PROTOCOL TOXICITY STUDIES	50%	24,030	
B	SPECIAL TOXICITY STUDIES	50%	24,030	
MARYLAND, UNIVERSITYOF (NM43748)				5,400,000
4X1	CLINICAL TRIALS - PHASE I	- 15% -	810,000	
B	PHARMACOLOGY/TOXICOLOGY	15%	810,000	
4X2	CLINICAL PHARMACOLOGY	- 10% -	540,000	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	10%	540,000	
5XX	CLINICAL TRIALS - PHASE II	- 15% -	810,000	
G	TESTICULAR	2%	108,000	
J	BRAIN	3%	162,000	
K	LYMPHOMAS	4%	216,000	
L	LEUKEMIA	3%	162,000	
N	OTHER DISEASES	3%	162,000	
6X1	CLINICAL TRIALS - PHASE III	- 15% -	810,000	
G	TESTICULAR	2%	108,000	
J	BRAIN	2%	108,000	
K	LYMPHOMAS	4%	216,000	
L	LEUKEMIA	4%	216,000	
N	OTHER DISEASES	3%	162,000	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
6X2	TOXICOLOGY/PHARMACOLOGY	- 10% -	540,000	
A	ANIMAL STUDIES	5%	270,000	
B	HUMAN STUDIES	5%	270,000	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	1,620,000	
G	TESTICULAR	3%	162,000	
J	BRAIN	4%	216,000	
K	LYMPHOMAS	8%	432,000	
L	LEUKEMIA	8%	432,000	
N	OTHER DISEASES	7%	378,000	
8XX	PROGRAM MANAGEMENT	- 5% -	270,000	
A	ADMINISTRATION	5%	270,000	
MAYO FOUNDATION (NM43783)			$	55,300
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
F	STOMACH	100%	55,300	
MAYO FOUNDATION (NM43796)				76,209
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E	PANCREAS	100%	76,209	
MAYO FOUNDATION (NM53772)				243,067
5XX	CLINICAL TRIALS - PHASE II	- 50% -	121,534	
A	BREAST	50%	121,534	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	121,533	
A	BREAST	50%	121,534	
MAYO FOUNDATION (NM53838)				165,715
4X1	CLINICAL TRIALS - PHASE I	- 100% -		
B	PHARMACOLOGY/TOXICOLOGY	100%	165,715	
MAYO FOUNDATION (NM02066)				100,000
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
B	COLON	90%	90,000	
E	PANCREAS	5%	5,000	
F	STOMACH	5%	5,000	
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES (NM43904)				161,700
4X1	CLINICAL TRIALS - PHASE I	- 100% -		
B	PHARMACOLOGY/TOXICOLOGY	100%	161,700	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
MERCK AND CO., INC. (NM22002)			$	402,065
2B4	PROC. OF PRECLINICAL MATERIALS	- 75% -	301,549	
A	SYNTHETICS PROCUREMENT	75%	301,549	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 25% -	100,516	
A	SYNTHETICS PRODUCTION	25%	100,516	
MIAMI, UNIV. OF (NM02225)				120,825
1XX	ACQUISITION OF MATERIALS	- 2% -	2,417	
E	BIOASSAY OF NATURAL PRODUCTS	2%	2,417	
2AX	BASIC SCREEN	- 98% -	118,408	
A	PRIMARY SCREENING IN VITRO	98%	118,409	
MIAMI, UNIV. OF (NM43800)				74,513
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E	PANCREAS	100%	74,513	
MICHIGAN CANCER FOUNDATION (NM53789)				55,089
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	55,089	
MICHIGAN DEPARTMENT DEPT. OF PUBLIC HEALTH (NM33702)				54,166
2AX	BASIC SCREEN	- 100% -		
A	PRIMARY SCREENING IN VITRO	100%	54,166	
MICRO. ASSOC. INC. (NB92177)				94,520
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
D	OTHER	100%	94,520	
MICRO. ASSOC., INC. (NM43793)				408,800
1XX	ACQUISITION OF MATERIALS	- 26% -	106,288	
A	SYNTHETICS PROCUREMENT	14%	57,232	
G	DATA PROCESSING AND SUPPORT	12%	49,056	
2AX	BASIC SCREEN	- 15% -	61,320	
B	PRIMARY SCREENING IN VIVO	13%	53,144	
D	ANIMALS	2%	8,176	
2B2	VERIFICATION SCREEN	- 44% -	179,872	
B	DETAILED DRUG EVALUATION IN VIVO	25%	102,200	
D	RELATED NEW ASSAY DEVELOPMENT	2%	8,176	
E	ANIMALS	3%	12,264	
G	OTHER	14%	57,232	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X1	PHARMACOLOGY/TOXICOLOGY	- 15% -	61,320	
C	ANIMALS	4%	16,352	
F	PHARMACOLOGY	11%	44,968	
MICRO.	ASSOC., INC. (NM43761)		$	374,345
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	374,345	
MICRO.	ASSOC.,INC. ASSOCIATES, INC. (NM33728)			858,630
1XX	ACQUISITION OF MATERIALS	- 26% -	223,247	
A	SYNTHETICS PROCUREMENT	14%	120,208	
G	DATA PROCESSING AND SUPPORT	12%	103,036	
2AX	BASIC SCREEN	- 15% -	128,794	
B	PRIMARY SCREENING IN VIVO	13%	111,622	
D	ANIMALS	2%	17,173	
2B2	VERIFICATION SCREEN	- 44% -	377,796	
B	DETAILED DRUG EVALUATION IN VIVO	25%	214,658	
D	RELATED NEW ASSAY DEVELOPMENT	2%	17,173	
E	ANIMALS	3%	25,759	
G	OTHER	14%	120,208	
3X1	PHARMACOLOGY/TOXICOLOGY	- 15% -	128,793	
C	ANIMALS	4%	34,345	
F	PHARMACOLOGY	11%	94,449	
MICROBIOLOGICAL	ASSOCIATES, INC. (NM53819)			158,900
1XX	ACQUISITION OF MATERIALS	- 14% -	22,246	
E	BIOASSAY OF NATURAL PRODUCTS	2%	3,178	
F	ANIMALS	12%	19,068	
2AX	BASIC SCREEN	- 63% -	100,107	
C	RELATED NEW ASSAY DEVELOPMENT	3%	4,767	
D	ANIMALS	60%	95,340	
2B2	VERIFICATION SCREEN	- 23% -	36,547	
E	ANIMALS	23%	36,547	
MINNESOTA,	UNIV. OF (NM43738)			67,017
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	67,017	
MISSOURI,	CURATORS OF THE UNIVERSITY OF (NM12323)			130,000
5XX	CLINICAL TRIALS - PHASE II	- 100% -		
Q	MARKERS	75%	97,500	
R	DATA PROCESSING AND SUPPORT	25%	32,500	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
MONSANTO RES.CORP. (NM23715)			$	219,994
284	PROC. OF PRECLINICAL MATERIALS	- 80% -	175,996	
E	RADIOLABELED MATERIALS PROCUREMENT	80%	175,995	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 20% -	43,998	
J	OTHER	20%	43,999	
MONSANTO RES.CORP. (NM33709)				346,295
284	PROC. OF PRECLINICAL MATERIALS	- 10% -	34,629	
B	PLANT PRODUCTS PROCUREMENT	10%	34,629	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 90% -	311,656	
B	PLANT PRODUCTS PRODUCTION	90%	311,657	
MOUNT SINAI SCHOOL OF MEDICINE (NM43703)				55,625
5XX	CLINICAL TRIALS - PHASE II	- 50% -	27,813	
M	OVARY	50%	27,813	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	27,812	
M	OVARY	50%	27,813	
MOUNT SINAI SCHOOL OF MEDICINE (NM53837)				116,000
4X1	CLINICAL TRIALS - PHASE I	- 100% -		
B	PHARMACOLOGY/TOXICOLOGY	100%	116,000	
MOUNT SINAI SCHOOL OF MEDICINE (NM53841)				150,782
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
F	STOMACH	100%	150,782	
MURPHY BREEDING LABS (NM40876)				5,805
1XX	ACQUISITION OF MATERIALS	- 14% -	813	
E	BIOASSAY OF NATURAL PRODUCTS	2%	116	
F	ANIMALS	12%	697	
2AX	BASIC SCREEN	- 63% -	3,657	
C	RELATED NEW ASSAY DEVELOPMENT	3%	174	
D	ANIMALS	60%	3,483	
2B2	VERIFICATION SCREEN	- 23% -	1,335	
D	RELATED NEW ASSAY DEVELOPMENT	3%	174	
E	ANIMALS	20%	1,161	
MURPHY BREEDING LABS (NM40875)				17,752
1XX	ACQUISITION OF MATERIALS	- 14% -	2,487	
E	BIOASSAY OF NATURAL PRODUCTS	2%	355	
F	ANIMALS	12%	2,130	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 63% -	11,183	
C	RELATED NEW ASSAY DEVELOPMENT	3%	533	
D	ANIMALS	60%	10,651	
2B2	VERIFICATION SCREEN	- 23% -	4,082	
D	RELATED NEW ASSAY DEVELOPMENT	3%	533	
E	ANIMALS	20%	3,550	
NATIONAL ACADEMY OF SCIENCES (ND52103)			$	12,850
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%	12,850	
NATIONAL ACADEMY OF SCIENCES (NM53850)				20,000
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%	20,000	
NATIONAL ACADEMY OF SCIENCES (NM53849)				22,000
8XX	PROGRAM MANAGEMENT	- 100% -		
B	DISSEMINATION OF INFORMATION	100%	22,000	
NATIONAL NAVAL MEDICAL CENTER (YM50100)				17,900
5XX	CLINICAL TRIALS - PHASE II	- 44% -	7,876	
A	BREAST	42%	7,518	
R	DATA PROCESSING AND SUPPORT	2%	358	
6X1	CLINICAL TRIALS - PHASE III	- 38% -	6,802	
A	BREAST	36%	6,444	
S	DATA PROCESSING AND SUPPORT	2%	358	
6X2	TOXICOLOGY/PHARMACOLOGY	- 2% -	358	
B	HUMAN STUDIES	2%	358	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 16% -	2,864	
A	BREAST	15%	2,685	
P	DATA PROCESSING AND SUPPORT	1%	179	
NAVAL WEAPONS CENTER (YM50101)				85,000
2B2	VERIFICATION SCREEN	- 100% -		
G	OTHER	100%	85,000	
NEW YORK UNIV. (NM71174)				41,962
5XX	CLINICAL TRIALS - PHASE II	- 30% -	12,559	
J	BRAIN	30%	12,559	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 70% -	29,303	
J	BRAIN	70%	29,303	
	NOTRE DAME, UNIV. OF (NM23718)			$ 35,000
2B2	VERIFICATION SCREEN	- 100% -		
G	OTHER	100%	35,000	
	OHIO STATE UNIV. (NM71175)			38,281
5XX	CLINICAL TRIALS - PHASE II	- 30% -	11,485	
J	BRAIN	30%	11,484	
6X1	CLINICAL TRIALS - PHASE III	- 70% -	26,796	
J	BRAIN	70%	26,797	
	OHIO STATE UNIV. (NM33736)			95,390
6X1	CLINICAL TRIALS - PHASE III	- 40% -	38,156	
C	LUNG	40%	38,156	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 60% -	57,234	
C	LUNG	60%	57,234	
	OREGON STATE UNIV. (NM23201)			100,309
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	100,309	
	PARKE, DAVIS AND (NM43754)			562,472
1XX	ACQUISITION OF MATERIALS	- 85% -	478,102	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	83%	466,852	
G	DATA PROCESSING AND SUPPORT	2%	11,249	
2AX	BASIC SCREEN	- 15% -	84,370	
A	PRIMARY SCREENING IN VITRO	10%	56,247	
C	RELATED NEW ASSAY DEVELOPMENT	5%	28,124	
	PARKE, DAVIS & CO. (NM23708)			327,139
2B4	PROC. OF PRECLINICAL MATERIALS	- 50% -	163,570	
A	SYNTHETICS PROCUREMENT	50%	163,570	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 50% -	163,569	
A	SYNTHETICS PRODUCTION	25%	81,785	
B	PLANT PRODUCTS PRODUCTION	25%	81,785	
	PENNSYLVANIA, UNIVERSITY OF (NM53830)			21,422
2AX	BASIC SCREEN	- 30% -	6,427	
C	RELATED NEW ASSAY DEVELOPMENT	30%	6,427	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN	- 70% -	14,995	
C MOLECULAR BIOLOGY PROGRAM	70%	14,995	
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (NM33719)		$	146,600
1XX ACQUISITION OF MATERIALS	- 100% -		
G DATA PROCESSING AND SUPPORT	100%	146,600	
PENNWALT CORP. (NM12309)			14,862
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	14,862	
PFIZER, INC. (NM53805)			198,991
1XX ACQUISITION OF MATERIALS	- 100% -		
H OTHER	100%	198,991	
PHARM-ECO (NM23707)			171,375
2B4 PROC. OF PRECLINICAL MATERIALS	- 40% -	68,550	
A SYNTHETICS PROCUREMENT	40%	68,550	
3X2 DRUG PROD. & FORM. FOR CLIN. TRIALS	- 60% -	102,825	
A SYNTHETICS PRODUCTION	60%	102,825	
PHILIPS ROXANE LABS.LABORATORIES, INC. (NM12139)			73,206
2B1 FORMULATION	- 20% -	14,642	
A DEVEL. OF EXPERIMENTAL FORMULATIONS	20%	14,641	
3X2 DRUG PROD. & FORM. FOR CLIN. TRIALS	- 80% -	58,564	
F FORMULATION	60%	43,924	
G ANALYTICAL AND QUALITY CONTROL	20%	14,641	
POMONA COLLEGE (NM33703)			41,700
1XX ACQUISITION OF MATERIALS	- 100% -		
H OTHER	100%	41,700	
PUBLIC HEALTH SERV. SERVICE (YM40003)			365,280
4X1 CLINICAL TRIALS - PHASE I	- 15% -	54,792	
B PHARMACOLOGY/TOXICOLOGY	15%	54,792	
4X2 CLINICAL PHARMACOLOGY	- 10% -	36,528	
A CLIN. PHARMACOLOGY & PHARMACOKINETICS	10%	36,528	
5XX CLINICAL TRIALS - PHASE II	- 15% -	54,792	
G TESTICULAR	2%	7,306	
J BRAIN	3%	10,958	
K LYMPHOMAS	4%	14,611	
L LEUKEMIA	3%	10,958	
N OTHER DISEASES	3%	10,958	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 15% -	54,792	
G	TESTICULAR	2%	7,306	
J	BRAIN	2%	7,306	
K	LYMPHOMAS	4%	14,611	
L	LEUKEMIA	4%	14,611	
N	OTHER DISEASES	3%	10,958	
6X2	TOXICOLOGY/PHARMACOLOGY	- 10% -	36,528	
A	ANIMAL STUDIES	5%	18,264	
B	HUMAN STUDIES	5%	18,264	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 30% -	109,584	
G	TESTICULAR	3%	10,958	
J	BRAIN	4%	14,611	
K	LYMPHOMAS	8%	29,222	
L	LEUKEMIA	8%	29,222	
N	OTHER DISEASES	7%	25,570	
8XX	PROGRAM MANAGEMENT	- 5% -	18,264	
A	ADMINISTRATION	5%	18,264	
RADIOBIOLOGICAL INSTITUTE (NM53763)				$ 57,023
2B2	VERIFICATION SCREEN	- 100% -		
A	DETAILED DRUG EVALUATION IN VITRO	20%	11,405	
B	DETAILED DRUG EVALUATION IN VIVO	80%	45,618	
RES. FDN OF STATE UNIVERSITY OF NEW NEW YORK (NM33715)				241,700
5XX	CLINICAL TRIALS - PHASE II	- 10% -	24,170	
F	DATA PROCESSING AND SUPPORT	10%	24,170	
6X1	CLINICAL TRIALS - PHASE III	- 25% -	60,425	
S	DATA PROCESSING AND SUPPORT	25%	60,425	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 35% -	84,595	
P	DATA PROCESSING AND SUPPORT	35%	84,595	
8XX	PROGRAM MANAGEMENT	- 30% -	72,510	
A	ADMINISTRATION	20%	48,340	
B	DISSEMINATION OF INFORMATION	10%	24,170	
RESEARCH TRIANGLE INST. (NM92019)				195,008
1XX	ACQUISITION OF MATERIALS	- 95% -	185,258	
B	PLANT PRODUCTS PROCUREMENT	75%	146,256	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	15%	29,251	
E	BIOASSAY OF NATURAL PRODUCTS	5%	9,750	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 5% -	9,750	
A PRIMARY SCREENING IN VITRO	5%	9,750	
RHODE ISLAND, UNIV. OF (NM43778)		$	43,917
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	43,917	
RHODE ISLAND, UNIV. OF (NM12312)			1,430
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	1,430	
SCRIPPS CLINIC & RES. FD. (NM22042)			12,000
1XX ACQUISITION OF MATERIALS	- 75% -	9,000	
A SYNTHETICS PROCUREMENT	75%	9,000	
2AX BASIC SCREEN	- 25% -	3,000	
A PRIMARY SCREENING IN VITRO	13%	1,560	
B PRIMARY SCREENING IN VIVO	12%	1,440	
SIDNEY FARBER CANCERCENTER (NM53839)			94,210
8X1 CLINICAL TRIALS - PHASE I	- 100% -		
B PHARMACOLOGY/TOXICOLOGY	100%	94,210	
SIMONSEN LABORATORIES (NM53847)			220,138
1XX ACQUISITION OF MATERIALS	- 14% -	30,821	
E BIOASSAY OF NATURAL PRODUCTS	7%	15,410	
F ANIMALS	7%	15,410	
2AX BASIC SCREEN	- 63% -	138,696	
D ANIMALS	53%	116,673	
F OTHER	10%	22,014	
2B2 VERIFICATION SCREEN	- 23% -	50,632	
E ANIMALS	20%	44,028	
G OTHER	3%	6,604	
SIMONSEN LABORATORIES (NM53817)			374,274
1XX ACQUISITION OF MATERIALS	- 14% -	52,399	
E BIOASSAY OF NATURAL PRODUCTS	2%	7,495	
F ANIMALS	12%	44,913	
2AX BASIC SCREEN	- 63% -	235,792	
C RELATED NEW ASSAY DEVELOPMENT	3%	11,228	
D ANIMALS	60%	224,564	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2B2	VERIFICATION SCREEN	- 23% -	86,093	
E	ANIMALS	23%	86,083	
SIMONSEN LABORATORIES (NM40883)			$	38,988
1XX	ACQUISITION OF MATERIALS	- 14% -	5,459	
E	BIOASSAY OF NATURAL PRODUCTS	2%	780	
F	ANIMALS	12%	4,679	
2AX	BASIC SCREEN	- 63% -	24,562	
C	RELATED NEW ASSAY DEVELOPMENT	3%	1,170	
D	ANIMALS	60%	23,393	
2B2	VERIFICATION SCREEN	- 23% -	8,967	
D	RELATED NEW ASSAY DEVELOPMENT	3%	1,170	
E	ANIMALS	20%	7,798	
SIMONSEN LABORATORIES (NM40884)				6,183
1XX	ACQUISITION OF MATERIALS	- 14% -	866	
F	BIOASSAY OF NATURAL PRODUCTS	2%	124	
F	ANIMALS	12%	742	
2AX	BASIC SCREEN	- 63% -	3,995	
C	RELATED NEW ASSAY DEVELOPMENT	3%	185	
D	ANIMALS	60%	3,710	
2B2	VERIFICATION SCREEN	- 23% -	1,422	
D	RELATED NEW ASSAY DEVELOPMENT	3%	195	
E	ANIMALS	20%	1,237	
SLOAN-KETTERING INSTITUTE FOR CANCERRESEARCH (NM53820)				74,884
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	74,884	
SMALL BUSINESS ADMINISTRATION (NM43719)				157,303
1XX	ACQUISITION OF MATERIALS	- 10% -	15,731	
H	OTHER	10%	15,730	
2B2	VERIFICATION SCREEN	- 90% -	141,572	
C	MOLECULAR BIOLOGY PROGRAM	30%	47,191	
D	RELATED NEW ASSAY DEVELOPMENT	60%	94,382	
SOUTHERN ANIM. FARMS (NM22013)				55,236
1XX	ACQUISITION OF MATERIALS	- 7% -	3,869	
F	ANIMALS	7%	3,967	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
2AX BASIC SCREEN	- 45% -	24,855	
C RELATED NEW ASSAY DEVELOPMENT	5%	2,762	
D ANIMALS	40%	22,094	
2B2 VERIFICATION SCREEN	- 48% -	26,512	
D RELATED NEW ASSAY DEVELOPMENT	5%	2,762	
E ANIMALS	43%	23,751	
		$	52,260
SOUTHERN ANIMAL FARMS (NM40888)			
1XX ACQUISITION OF MATERIALS	- 14% -	7,318	
E BIOASSAY OF NATURAL PRODUCTS	2%	1,045	
F ANIMALS	12%	6,271	
2AX BASIC SCREEN	- 63% -	32,923	
C RELATED NEW ASSAY DEVELOPMENT	3%	1,568	
D ANIMALS	60%	31,356	
2B2 VERIFICATION SCREEN	- 23% -	12,019	
D RELATED NEW ASSAY DEVELOPMENT	3%	1,568	
E ANIMALS	20%	10,452	
			11,900
SOUTHERN ANIMAL FARMS (NM40889)			
1XX ACQUISITION OF MATERIALS	- 14% -	1,666	
E BIOASSAY OF NATURAL PRODUCTS	2%	238	
F ANIMALS	12%	1,428	
2AX BASIC SCREEN	- 63% -	7,497	
C RELATED NEW ASSAY DEVELOPMENT	3%	357	
D ANIMALS	60%	7,140	
2B2 VERIFICATION SCREEN	- 23% -	2,737	
D RELATED NEW ASSAY DEVELOPMENT	3%	357	
E ANIMALS	20%	2,380	
			$25,763
SOUTHERN ANIMAL FARMS (NM53845)			
1XX ACQUISITION OF MATERIALS	- 11% -	13,836	
E BIOASSAY OF NATURAL PRODUCTS	2%	2,515	
F ANIMALS	9%	11,310	
2AX BASIC SCREEN	- 52% -	65,395	
C RELATED NEW ASSAY DEVELOPMENT	3%	3,773	
D ANIMALS	49%	61,624	
2B2 VERIFICATION SCREEN	- 36% -	45,274	
D RELATED NEW ASSAY DEVELOPMENT	5%	6,288	
E ANIMALS	31%	38,987	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X1 PHARMACOLOGY/TOXICOLOGY	- 1% -	1,257	
C ANIMALS	1%	1,258	
SOUTHERN CAL., UNIV.UNIVERSITY OF (NM43795)		$	70,259
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E PANCREAS	100%	70,259	
SOUTHERN CALIF. UNIVERSITY OF (NM23241)			59,278
6X2 TOXICOLOGY/PHARMACOLOGY	- 100% -		
A ANIMAL STUDIES	75%	44,459	
C DATA PROCESSING AND SUPPORT	25%	14,820	
SOUTHERN CALIFORNIA,UNIVERSITY OF (NM53842)			143,500
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
F STOMACH	100%	143,500	
SOUTHERN RES.INST. (NM02083)			151,809
3X1 PHARMACOLOGY/TOXICOLOGY	- 40% -	60,724	
B SPECIAL TOXICITY STUDIES	40%	60,724	
6X2 TOXICOLOGY/PHARMACOLOGY	- 60% -	91,085	
A ANIMAL STUDIES	60%	91,085	
SOUTHERN RESEARCH INSTITUTE (NM43780)			494,353
3X1 PHARMACOLOGY/TOXICOLOGY	- 85% -	420,201	
D DATA PROCESSING AND SUPPORT	10%	49,435	
F PHARMACOLOGY	75%	370,765	
6X2 TOXICOLOGY/PHARMACOLOGY	- 15% -	74,152	
A ANIMAL STUDIES	15%	74,153	
SOUTHERN RESEARCH INSTITUTE (NM43762)			624,333
1XX ACQUISITION OF MATERIALS	- 100% -		
A SYNTHETICS PROCUREMENT	100%	624,333	
SOUTHERN RESEARCH INSTITUTE (NM43784)			372,473
2B2 VERIFICATION SCREEN	- 100% -		
C MOLECULAR BIOLOGY PROGRAM	100%	372,473	
SPARTAN RES. ANIM., INC. (NM30858)			463
1XX ACQUISITION OF MATERIALS	- 14% -	66	
E BIOASSAY OF NATURAL PRODUCTS	2%	9	
F ANIMALS	12%	56	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
2AX	BASIC SCREEN	- 63% -	291	
C	RELATED NEW ASSAY DEVELOPMENT	3%	14	
D	ANIMALS	60%	279	
2B2	VERIFICATION SCREEN	- 23% -	106	
D	RELATED NEW ASSAY DEVELOPMENT	3%	14	
E	ANIMALS	20%	93	
STANFORD RES.INST. (NM12162)				$ 209,990
2B4	PROC. OF PRECLINICAL MATERIALS	- 80% -	167,992	
E	RADIOLABELED MATERIALS PROCUREMENT	80%	167,992	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 20% -	41,998	
J	OTHER	20%	41,998	
STANFORD RES.INST. (NM23200)				93,923
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	93,923	
STARKS ASSOC., INC. INC. (NM23203)				343,730
2B4	PROC. OF PRECLINICAL MATERIALS	- 60% -	206,238	
A	SYNTHETICS PROCUREMENT	60%	206,238	
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 40% -	137,492	
A	SYNTHETICS PRODUCTION	40%	137,492	
STARKS, C. P., INC. (NM53745)				348,579
1XX	ACQUISITION OF MATERIALS	- 100% -		
A	SYNTHETICS PROCUREMENT	100%	348,579	
TACONIC FARMS (NM40864)				29,700
1XX	ACQUISITION OF MATERIALS	- 14% -	4,158	
E	BIOASSAY OF NATURAL PRODUCTS	2%	594	
F	ANIMALS	12%	3,564	
2AX	BASIC SCREEN	- 63% -	18,711	
C	RELATED NEW ASSAY DEVELOPMENT	3%	891	
D	ANIMALS	60%	17,820	
2B2	VERIFICATION SCREEN	- 23% -	6,831	
D	RELATED NEW ASSAY DEVELOPMENT	3%	891	
E	ANIMALS	20%	5,940	
TENNESSEE, UNIV. OF (NM02084)				80,000
2B1	FORMULATION	- 25% -	20,000	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	25%	20,000	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 75% -	60,000	
F	FORMULATION	65%	52,000	
G	ANALYTICAL AND QUALITY CONTROL	10%	8,000	
TEXAS INBRED MICE (NM43711)				$ 42,550
1XX	ACQUISITION OF MATERIALS	- 14% -	5,958	
E	BIOASSAY OF NATURAL PRODUCTS	2%	851	
F	ANIMALS	12%	5,106	
2AX	BASIC SCREEN	- 63% -	26,806	
C	RELATED NEW ASSAY DEVELOPMENT	3%	1,277	
D	ANIMALS	60%	25,530	
2B2	VERIFICATION SCREEN	- 23% -	9,786	
E	ANIMALS	23%	9,787	
TEXAS INBRED MICE (NM40927)				15,840
1XX	ACQUISITION OF MATERIALS	- 14% -	2,218	
E	BIOASSAY OF NATURAL PRODUCTS	2%	317	
F	ANIMALS	12%	1,901	
2AX	BASIC SCREEN	- 63% -	9,979	
C	RELATED NEW ASSAY DEVELOPMENT	3%	475	
D	ANIMALS	60%	9,504	
2B2	VERIFICATION SCREEN	- 23% -	3,643	
D	RELATED NEW ASSAY DEVELOPMENT	3%	475	
E	ANIMALS	20%	3,168	
TEXAS INBRED MICE (NM53816)				468,053
1XX	ACQUISITION OF MATERIALS	- 14% -	65,528	
E	BIOASSAY OF NATURAL PRODUCTS	2%	9,361	
F	ANIMALS	12%	56,166	
2AX	BASIC SCREEN	- 63% -	294,873	
C	RELATED NEW ASSAY DEVELOPMENT	3%	14,042	
D	ANIMALS	60%	280,832	
2B2	VERIFICATION SCREEN	- 23% -	107,652	
E	ANIMALS	23%	107,652	
TEXAS, UNIV. OF OF, SYSTEM CANCER CTR/MDA (NM43786)				81,586
2B2	VERIFICATION SCREEN	- 100% -		
C	MOLECULAR BIOLOGY PROGRAM	100%	81,586	
TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA (NM43801)				247,800
4X1	CLINICAL TRIALS - PHASE I	- 100% -		
B	PHARMACOLOGY/TOXICOLOGY	100%	247,800	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT		DOLLAR LEVEL
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM61156)			$	23,000
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	11,500	
F	PHARMACOLOGY	50%	11,500	
4X2	CLINICAL PHARMACOLOGY	- 10% -	2,300	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	10%	2,300	
6X2	TOXICOLOGY/PHARMACOLOGY	- 40% -	9,200	
A	ANIMAL STUDIES	10%	2,300	
B	HUMAN STUDIES	30%	6,900	
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33710)				203,233
5XX	CLINICAL TRIALS - PHASE II	- 70% -	142,264	
M	OVARY	70%	142,263	
6X1	CLINICAL TRIALS - PHASE III	- 30% -	60,969	
M	OVARY	30%	60,970	
TEXAS, UNIVERSITY OF, M.D. ANDERSON (NM33737)				376,836
5XX	CLINICAL TRIALS - PHASE II	- 20% -	75,368	
C	LUNG	20%	75,367	
6X1	CLINICAL TRIALS - PHASE III	- 40% -	150,734	
C	LUNG	40%	150,734	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 40% -	150,734	
C	LUNG	40%	150,734	
TEXAS, UNIVERSITY OF(NM53832)				550,000
6X1	CLINICAL TRIALS - PHASE III	- 100% -		
P	PROTECTED ENVIRONMENT	100%	550,000	
TULANE UNIVERSITY (NM53815)				179,985
1XX	ACQUISITION OF MATERIALS	- 14% -	25,199	
E	BIOASSAY OF NATURAL PRODUCTS	2%	3,600	
F	ANIMALS	12%	21,593	
2AX	BASIC SCREEN	- 63% -	113,390	
C	RELATED NEW ASSAY DEVELOPMENT	3%	5,400	
D	ANIMALS	60%	107,991	
2B2	VERIFICATION SCREEN	- 23% -	41,396	
F	ANIMALS	23%	41,397	
TULANE UNIVERSITY (NM33701)				24,956
2B2	VERIFICATION SCREEN	- 95% -	23,708	
D	RELATED NEW ASSAY DEVELOPMENT	5%	1,248	
E	ANIMALS	90%	22,460	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
3X1	PHARMACOLOGY/TOXICOLOGY	- 5% -	1,247	
C	ANIMALS	5%	1,248	
UPJOHN COMPANY (NM43753)				$ 1,248,400
1XX	ACQUISITION OF MATERIALS	- 57% -	711,588	
C	FERMENTATION/ANTIBIOTICS PROCUREMENT	55%	686,620	
G	DATA PROCESSING AND SUPPORT	2%	24,968	
2AX	BASIC SCREEN	- 17% -	212,228	
A	PRIMARY SCREENING IN VITRO	7%	87,388	
B	PRIMARY SCREENING IN VIVO	3%	37,452	
C	RELATED NEW ASSAY DEVELOPMENT	7%	87,388	
2B1	FORMULATION	- 1% -	12,484	
A	DEVEL. OF EXPERIMENTAL FORMULATIONS	1%	12,494	
2B2	VERIFICATION SCREEN	- 10% -	124,840	
C	MOLECULAR BIOLOGY PROGRAM	10%	124,840	
4X2	CLINICAL PHARMACOLOGY	- 15% -	187,260	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	13%	162,292	
B	DATA PROCESSING AND SUPPORT	2%	24,968	
UTAH, UNIV. OF (NM43806)				214,233
1XX	ACQUISITION OF MATERIALS	- 82% -	175,672	
A	SYNTHETICS PROCUREMENT	82%	175,671	
2AX	BASIC SCREEN	- 18% -	38,561	
A	PRIMARY SCREENING IN VITRO	18%	38,562	
VALUE ENGINEERING COMPANY (NM53821)				71,123
3X2	DRUG PROD. & FORM. FOR CLIN. TRIALS	- 100% -		
H	DATA PROCESSING AND SUPPORT	100%	71,123	
VALUE ENGINEERING COMPANY (NM33706)				829,980
1XX	ACQUISITION OF MATERIALS	- 14% -	116,198	
G	DATA PROCESSING AND SUPPORT	14%	116,197	
2AX	BASIC SCREEN	- 60% -	497,988	
E	DATA PROCESSING AND SUPPORT	60%	497,988	
2B2	VERIFICATION SCREEN	- 26% -	215,794	
F	DATA PROCESSING AND SUPPORT	26%	215,795	
VERMONT, UNIV. OF (NM33716)				31,337
5XX	CLINICAL TRIALS - PHASE II	- 50% -	15,669	
P	DATA PROCESSING AND SUPPORT	50%	15,669	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X1	CLINICAL TRIALS - PHASE III	- 50% -	15,669	
S	DATA PROCESSING AND SUPPORT	50%	15,669	
	VETERANS ADMIN. (YM40004)			$ 2,622,000
4X1	CLINICAL TRIALS - PHASE I	- 10% -	262,200	
B	PHARMACOLOGY/TOXICOLOGY	10%	262,200	
5XX	CLINICAL TRIALS - PHASE II	- 20% -	524,400	
B	COLON	1%	26,220	
C	LUNG	15%	393,300	
E	PANCREAS	1%	26,220	
F	STOMACH	1%	26,220	
H	PROSTATE	2%	52,440	
6X1	CLINICAL TRIALS - PHASE III	- 50% -	1,311,000	
B	COLON	3%	78,650	
C	LUNG	35%	917,700	
E	PANCREAS	4%	104,880	
F	STOMACH	3%	78,650	
H	PROSTATE	5%	131,100	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 20% -	524,400	
B	COLON	2%	52,440	
C	LUNG	15%	393,300	
E	PANCREAS	2%	52,440	
F	STOMACH	1%	26,220	
	VIRGINIA, UNIV.OF (NM12099)			190,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
D	PLANT PRODUCTS PROCUREMENT	100%	180,000	
	WALTER REED ARMY MED.CENTER(YM40005)			34,247
4X2	CLINICAL PHARMACOLOGY	- 2% -	688	
A	CLIN. PHARMACOLOGY & PHARMACOKINETICS	2%	685	
5XX	CLINICAL TRIALS - PHASE II	- 46% -	15,753	
A	BREAST	40%	13,699	
C	LUNG	1%	342	
H	PROSTATE	2%	695	
J	BRAIN	2%	685	
R	DATA PROCESSING AND SUPPORT	1%	342	
6X1	CLINICAL TRIALS - PHASE III	- 29% -	9,931	
A	BREAST	20%	6,849	
G	TESTICULAR	8%	2,740	
S	DATA PROCESSING AND SUPPORT	1%	342	

	CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
6X2	TOXICOLOGY/PHARMACOLOGY	- 2% -	684	
B	HUMAN STUDIES	2%	685	
7XX	CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 21% -	7,191	
A	BREAST	20%	6,849	
P	DATA PROCESSING AND SUPPORT	1%	342	
	WARF INSTITUTE, INC.(NM43707)			$ 102,682
1XX	ACQUISITION OF MATERIALS	- 100% -		
B	PLANT PRODUCTS PROCUREMENT	100%	102,682	
	WASH. UNIV. UNIVERSITY SCHOOL OFMEDICINE (NM43731)			106,085
2B2	VERIFICATION SCREEN	- 100% -		
A	DETAILED DRUG EVALUATION IN VITRO	25%	26,521	
B	DETAILED DRUG EVALUATION IN VIVO	75%	79,564	
	WASH STATE UNIV. UNIVERSITY (NM22059)			36,729
1XX	ACQUISITION OF MATERIALS	- 14% -	5,143	
E	BIOASSAY OF NATURAL PRODUCTS	4%	1,469	
F	ANIMALS	10%	3,673	
2AX	BASIC SCREEN	- 63% -	23,139	
C	RELATED NEW ASSAY DEVELOPMENT	3%	1,102	
D	ANIMALS	60%	22,037	
2B2	VERIFICATION SCREEN	- 23% -	8,447	
D	RELATED NEW ASSAY DEVELOPMENT	3%	1,102	
E	ANIMALS	20%	7,346	
	WASHINGTON HOSPITAL CENTER (NM43710)			37,000
6X2	TOXICOLOGY/PHARMACOLOGY	- 100% -		
A	ANIMAL STUDIES	100%	37,000	
	WISCONSIN,BOARD OF REGENTS OF THE UNIVERSITY OF (NM33732)			40,000
1XX	ACQUISITION OF MATERIALS	- 100% -		
E	BIOASSAY OF NATURAL PRODUCTS	100%	40,000	
	WISCONSIN,UNIV.OF (NM12275)			95,000
1XX	ACQUISITION OF MATERIALS	- 50% -	47,500	
A	SYNTHETICS PROCUREMENT	50%	47,500	
3X1	PHARMACOLOGY/TOXICOLOGY	- 50% -	47,500	
F	PHARMACOLOGY	50%	47,500	

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
YALE UNIV. (NM43797)		$	75,088
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
E PANCREAS	100%	75,088	
YALE UNIVERSITY (NM53843)			102,200
7XX CLIN. TRIALS - PHASE IV (COMB. MODAL)	- 100% -		
F STOMACH	100%	102,200	
YALE UNIVERSITY SCHOOL OF MEDICINE (NM53824)			58,605
1XX ACQUISITION OF MATERIALS	- 50% -	29,303	
A SYNTHETICS PROCUREMENT	50%	29,303	
2B2 VERIFICATION SCREEN	- 50% -	29,302	
C MOLECULAR BIOLOGY PROGRAM	50%	29,303	
YALE UNIVERSITY (NM12339)			84,000
1XX ACQUISITION OF MATERIALS	- 75% -	63,000	
A SYNTHETICS PROCUREMENT	75%	63,000	
2AX BASIC SCREEN	- 25% -	21,000	
A PRIMARY SCREENING IN VITRO	12%	10,080	
B PRIMARY SCREENING IN VIVO	13%	10,920	
YALE UNIVERSITY SCHOOL OF MEDICINE (NM33711)			26,378
2AX BASIC SCREEN	- 67% -	17,674	
A PRIMARY SCREENING IN VITRO	67%	17,673	
4X1 CLINICAL TRIALS - PHASE I	- 16% -	4,220	
B PHARMACOLOGY/TOXICOLOGY	16%	4,220	
4X2 CLINICAL PHARMACOLOGY	- 17% -	4,494	
A CLIN. PHARMACOLOGY & PHARMACOKINETICS	17%	4,494	
YESHIVA UNIV. (NM43787)			82,800
2B2 VERIFICATION SCREEN	- 100% -		
C MOLECULAR BIOLOGY PROGRAM	100%	82,800	

TABLE IV
DESCRIPTION OF CONTRACTS IN THE DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (NO1-CM-43704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. The Contractor supplies large amounts of active, purified conditioning factor that will be considered acceptable for clinical trials. The material will have potential clinical use in leukemia, aplastic anemia and drug-induced aplasias.

ABCOR, INC. (NO1-CM-02239)

This contract provided engineering investigations and development to significantly improve the yields of human blood cells, particularly leukocytes, from hematologically normal donors, (1) through modifications of the NCI-IBM Blood Cell Separator or its operational techniques, or (2) through entirely new systems of procurement based upon different principles of blood cell separation such as centrifugal elutriation, filtration leukapheresis, etc. This contract expired October 1974.

AEROJET-GENERAL CORPORATION (NO1-CM-81300)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS - YO1-CM-40001)

Under the Transfer of Funds agreement with the Medicinal Plant Resources Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections are shipped to WARF Institute, Inc. for extraction and the extracts are screened *in vitro* and *in vivo* for antineoplastic activity. Confirmed active plants are recollected in large quantities on a priority based on the needs of the Division of Cancer Treatment Program for shipment to the fractionating chemists. Very large recollections are made, as requested by Drug Research and Development, for the preparation of clinical quantities of drugs. Large collections are made of species, not previously screened, that are members of the families known to be rich sources of activity. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALBANY MEDICAL COLLEGE (NO1-CM-22074)

This contract was designed to evaluate the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival. A study comparing CCNU and 5-Azacytidine was completed with 2/21 regressions, and 2/19 with CCNU. Thirty-two patients were entered by Albany comparing Adriamycin with adrenalectomy or combination of Cytoxan, 5-Fluorouracil and Prednisone (CFP). An additional 46 patients were entered on this study by Roswell Park. Among the evaluable patients there were responses in 8/22 with Adriamycin, 11/25 with CFP and 6/18 with adrenalectomy.

ALDRICH CHEMICAL COMPANY (NO1-CM-23706)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

ALLEGHENY GENERAL HOSPITAL (NO1-CM-43730)

The objective of this contract is to determine the dose and time course effects of antitumor drugs selected by the National Cancer Institute on survival of normal gastrointestinal crypts and on the *in vivo* survival of experimental murine mastocytoma cells. The effects of multiple drug dose interval on the normal gastrointestinal mucosa and the mastocytoma (in various metastatic sites) will be evaluated. Information on dose schedules and drug combinations leading to minimal or maximal gastrointestinal toxicity will be provided concurrent with the effects of these therapies on a proliferating murine mastocytoma. The methods used in these studies to determine gastrointestinal crypt survival were developed by the Contractor.

ARIZONA BOARD OF REGENTS (ARIZONA STATE UNIVERSITY) (NO1-CM-12308)

The major objective of this contract is the preparation, from animal, insect and plant materials including marine species, of extracts suitable for bioassay in the National Cancer Institute antitumor screen. The raw natural product specimens are obtained in most instances by the Contractor. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute and in amounts suitable for routine *in vivo* screening. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation.

ARIZONA BOARD OF REGENTS (UNIVERSITY OF ARIZONA) (NO1-CM-33750)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Recent promising results have been obtained from the extraction of plants collected in Greece, Hawaii and Arizona. Further fractionation is being carried out to isolate the active components. A KB cell culture unit has been added to expedite the fractionation work.

ARIZONA, UNIVERSITY OF (NO1-CM-33713)

The third year of this contract (begun November 10, 1973) was devoted to the further development of a staging system for patients with multiple myeloma. The rationale for this proposed staging system was based on detailed studies by the Principal Investigator, Dr. Sydney Salmon, in working out a procedure for determining total myeloma cell number in patients. The proposed system incorporates available clinical and laboratory data and measurements in conjunction with tumor cell number to derive a means for staging myeloma patients. This system is now being tested prospectively to determine if the desired correlations with actual tumor cell measurements hold true. It is anticipated this system could be used for evaluation of the number of tumor cells remaining following therapy using clinical parameters without the necessity for carrying out the detailed and involved procedures for actual measurement of this number.

ARS/SPRAGUE-DAWLEY (NO1-CM-02044)

This rodent production center contract supported a production effort designed to furnish animals in accordance with laboratory requirements. Breeding animals were furnished by the Government. Major emphasis was directed toward AKR mouse production. This contract terminated October 1974.

ARS/SPRAGUE-DAWLEY (NO1-CM-12342)

This contract provided for the maintenance of a rodent production center. This center produced stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies were correlated with requirements for specific investigations. This contract also furnished breeding animals for large-scale production colonies. Major emphasis was upon the production of AKR mice. This contract terminated January 1975.

ARS/SPRAGUE-DAWLEY (NO1-CM-53813) (FORMERLY NO1-CM-12007)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct tasks identified as follows:

Task 1 provides inbred, outbred and hybrid rodents in modified conventional environments.

ARS/SPRAGUE-DAWLEY (N01-CM-53813 (FORMERLY N01-CM-12007) (CONTINUED)

Task 2 provides Syrian golden hamsters with defined but non-pathogenic organisms. A small percentage of the effort is devoted to the development of germ-free hamsters.

Task 3 provides inbred and outbred laboratory strains of rats as germ-free and/or with defined microflora.

Task 4 provides various germ-free inbred, outbred and hybrids of inbred mice and the production of counterparts of the various species with defined microflora.

ARS/SPRAGUE-DAWLEY (N01-CM-53846)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of AKR mice.

ARTHUR D. LITTLE, INC. (N01-CM-23711)

This contract provides broad biomedical engineering resources which are responsive to the support of individual research projects within the Division of Cancer Treatment. A flexible mechanism of support defines and implements engineering projects in close collaboration with the Division of Cancer Treatment staff in active areas such as: automated microspectometry, cell kinetics modeling, the automation of grain counting and bone marrow colony assays and studies of the protective laminar flow environment for supportive care of leukemic patients. A "core group" of senior engineers continually consults and interacts with the National Cancer Institute staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under National Cancer Institute staff direction.

ARTHUR D. LITTLE, INC. (N01-CM-33727)

Tasks conducted under this multifaceted contract were divided into the following major components during the past year. As a consequence of changes in DCT program emphasis and the recompetition of specific areas of research, some of these tasks are now carried out under other contracts or have been terminated. These are noted specifically.

The Screening and Experimental Therapeutics and Tumor Biology effort provides for routine primary screening *in vivo* of synthetic and natural products, the detailed evaluation of active drugs, schedule dependency and combination chemotherapy studies and the development of new and improved methods for *in vivo* screening and detailed drug evaluation. Considerable emphasis is placed

ARTHUR D. LITTLE, INC. (NO1-CM-33727) (CONTINUED)

on the study of combination chemotherapy and the combination of drugs plus radiation. This task was conducted under this contract for a part of FY-1975. The primary and secondary screening effort and detailed drug evaluation in vivo are currently carried out under contract NO1-CM-57006 (Arthur D. Little, Inc.). The combination chemotherapy and combined modality studies are currently carried out under contract NO1-CM-53765 (Arthur D. Little, Inc.).

Physiological Disposition and Biochemical Pharmacology studies on drugs of interest to the Division of Cancer Treatment were conducted under this contract for two months of the contract year. These studies are currently conducted under contract NO1-CM-53849. Similarly, the Electron Microscopy and Radioautography effort providing such services in support of in vivo and in vitro studies were carried out under this contract for only a portion of FY-1975 and are currently conducted under contract NO1-CM-53849.

Maintenance of the Division of Cancer Treatment's primary Animal Tumor Bank and Tumor Distribution effort provided for the distribution of standard tumor lines to contract screening laboratories and other research institutions. This task also included methodological studies on the frozen, stored tumor and cell lines. As a result of recompetition, this bank was expanded to include tumor and cell lines previously carried at another laboratory. This task is no longer carried out under this contract and is currently conducted under contract NO1-CM-53849.

The Applied Mathematical and Computer Oriented services provide for the analysis of studies from all appropriate contract laboratories related to the development and finalization of new protocols for screening. The principal objective is to advise Drug Research and Development regarding experimental design, parameters and criteria for activity and data analysis. The contract segment also conducts studies of correlations between clinical activity and activity in animal tumor assays and advises program with respect to relative, predictive value among alternative animal systems.

The Cell Culture and Related Methodology work consists of routine in vitro screening, bioassay of natural product fractions, detailed drug evaluation in vitro, and the development of improved assays for natural product bioassay.

The Central Nervous System solid tumor studies are concerned with the development and evaluation of transplantable gliomas in the CNS of beagle dogs, together with collaborative studies with clinical and pharmacological groups of the National Cancer Institute to examine the growth kinetics and the behavior of transplantable gliomas to selected groups. This effort will be recompeted within the next year.

The Neuropharmacology studies were aimed at approaching various toxicological and pharmacological problems relating to the treatment of neoplastic diseases of the Central Nervous System. This segment has been terminated.

ARTHUR D. LITTLE, INC. (NO1-CM-53765)

This contract involves in vivo primary screening of structural congeners of known active drugs, studies of drug combinations of potential clinical interest, and combined modality studies (drugs plus radiation or surgery). The drug "analog" screening segment differs from the routine primary screening of "random" compounds in that the former utilizes host-tumor systems and experimental protocols selected on the basis of the known biological, biochemical and pharmacological characteristics of the parent drug. Results are closely integrated with in vitro studies carried out under contract NO1-CM-33727 (Arthur D. Little, Inc.) and physiological disposition and biochemistry studies carried out under contract NO1-CM-53849 (Arthur D. Little, Inc.). This integrated program provides for comprehensive structure-activity relationships for structural classes of interest to the Division of Cancer Treatment. Most of the compounds studied are synthesized under the Division of Cancer Treatment's sponsored contracts. In order to aid in the determination of promising leads for further synthesis, results of in vivo testing are transmitted directly and rapidly from this Contractor to the contract synthetic chemist. The combination chemotherapy and combined modality segment of this contract evaluates the potential superiority of such treatments over single modality therapy and utilizes appropriate in vivo experimental systems. Studies are generally restricted to drug combinations and combined modalities of current clinical interest. Emphasis is placed on assessing the influence of factors that are known to modify combined treatment, e.g., the dose ratio between two drugs, the treatment schedule, the sequence of treatment courses, and the interval between the administration of two drugs or between surgery or radiation and drug treatment.

ARTHUR D. LITTLE, INC. (NO1-CM-57006)

This is a service contract for the in vivo screening of new synthetic and natural product materials and the follow-up testing of active materials. The primary screening is carried out in accordance with Drug Research and Development protocols which require new materials to be tested against either the L1210 or the P388 leukemia test systems. The current level of testing is approximately 25,000 L1210 equivalent tests per year. As requested, detailed testing is performed with other tumor test systems, such as the Lewis lung carcinoma and the B16 melanoma, and schedule dependency studies conducted with materials of potential clinical interest. Methodology studies, such as the characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer.

ASSOCIATED BIOMEDIC SYSTEMS (NO1-CM-02271)

The purposes of this contract are: (1) to supply normal human blood lymphocytes, both untreated and stimulated with phytohemagglutinin; (2) to produce and evaluate human myeloid colony-stimulating factor(s) by "conditioning" media with various cells and then testing such media for "factor" activity; (3) to perform various services regarding culture of human cell lines such as (a) mass production of "interesting" lines (e.g., cells containing RNA-directed DNA polymerase, DNA polymerase γ, terminal deoxynucleotidyl transferase, or cell producing "factors"), and (b) ridding cell lines of contaminated mycoplasma by various techniques. About 250 billion lymphocytes were provided last year. Also, 500 billion cultured lymphoblastoid cells were provided. Certain cell-free "conditioned" media have been identified as rich sources of colony-stimulating factor. At least one cell line has been freed of detectable mycoplasma.

ATOMIC ENERGY COMMISSION (Y01-CM-10056)

The principal objective of this project is to study the cell cycle phase specificity of clinical drugs and drugs in development toward clinical trials. Drugs are evaluated for their ability to selectively inhibit progress through G_1, S, G_2 and M- phases of their proliferative cycle and for their selective cytocidal effects in these phases, as well. Both synchronized and exponentially growing mammalian cell culture systems are used. The importance of such studies stems from the need to identify and develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic cells. The *in vitro* methodologies used were developed by the Contractor.

ATOMIC ENERGY COMMISSION (Y01-CM-10060)

This contract involves the study of potential biologic markers for cancer in body fluids by means of high resolution high-pressure ion exchange chromatography. Initial efforts have focused on minor and methylated nucleic acid derivatives (tRNA catabolic end products) in urine. Sensitive analytical tools utilized in addition to high-pressure chromatography include gas chromatography, gas chromatography-mass spectrometry, and high- and low-resolution mass spectrometry. Preliminary quantitative results indicated elevated levels for specific nucleosides in the urine of approximately 60% of the cancer patients studied. Further efforts are focused on the analysis of specific serum protein bound sugars (utilizing high-pressure liquid chromatographic separation) in the blood of patients with malignancies. In addition, studies are in progress to determine the compounds represented by abnormal peaks obtained on chromatograms from patients with various selected types of cancer.

ATOMIC ENERGY COMMISSION (Y01-CM-40101)

The objective of this effort is to develop a blood cell separation method which will collect sufficient granulocytes ($\sim 10^{10}$/day) from normal donors to permit widespread adoption of this technique in granulocyte replacement therapy. A unique design approach which was the end result of an extensive ORNL analysis "Blood Cell Separator Development for In Vivo Leukapheresis" is being followed. This consists of radically modifying the blood flow path of the existing IBM rotor to mechanically enhance rouleax formation and to photoelectrically monitor and control separating cell fractions in the interior of the rotor. This feasibility effort covers rotor fabrication and testing with dog models.

ATOMIC ENERGY COMMISSION (Y01-CM-40102)

This agreement will accomplish R&D with instrumental and biological techniques to progressively adapt a prototype cell microfluorometer and computer data analysis system for human tumor cell characterization and drug evaluation studies planned by the National Cancer Institute. The initial year will accomplish the engineering, fabrication, testing and delivery of a multiparameter cell microfluorometer and sorting device coupled with a PDP-11/40 computer system as well as interim interface device for data collection and analysis. Also to be conducted in parallel are biological studies concerning "instrument compatible" cell staining, dispersal and fixation techniques on untested cell samples from a variety of human and animal tumors of current National Cancer Institute interest. During the second year, it is planned to continue the cell preparation work to explore and optimize techniques, and to exploit the instrument's potential for characterizing model animal tumors (L1210 ascites, B16 melanoma, Lewis lung carcinoma) as well as several human tumors both *in vivo* and *in vitro*.

AUTOMATION INDUSTRIES, INC. (N01-CM-33717)

This contract provides support for the Medical Oncology Area in the collection and computer processing of clinical data. Contractor extracts data from medical records and other clinical sources and assists in the development and maintenance of computer files, and the generation of computer retrievals and reports. During the past year, the Contractor has demonstrated excellent capabilities in the accurate extraction and computerization of medical data.

AUTOMATION INDUSTRIES, INC. (N01-CM-33732)

This service contract was established to provide the necessary administrative and technical support to the Office of the Director, Division of Cancer Treatment. The primary function is the coordination and technical skills involved with the organization of conferences, graphic support and editorial services.

BATTELLE-INSTITUT, E.V. (N01-CM-57008)

This contract provides for the primary *in vivo* screening of new materials and the detailed evaluation of those indicating preliminary activity. The current level of testing is approximately 25,000 L1210 equivalent tests per year. All testing is carried out in accordance with the Drug Research and Development protocols which currently require new materials to be tested against the L1210 or the P388 leukemia test systems. As requested by Drug Research and Development staff, compounds of interest are tested against other test systems as the Lewis lung carcinoma and the B16 melanoma and schedule dependency studies to determine optimum route and regimen are carried out. Methodology is performed as requested by the Project Officer.

BATTELLE MEMORIAL INSTITUTE (N01-CM-02008)

The Contractor performed bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimens were forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples were screened per year. The research portion of this contract (a minor component) was directed towards improvement in cultural techniques and the elucidation of salmonella inhibitory substances associated with certain microorganisms. This contract terminated February 1975.

BATTELLE MEMORIAL INSTITUTE (N01-CM-43746)

In March 1974 a Prime Contract was awarded to Battelle Memorial Institute for supervision of all contracts carrying out the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the prime contract mechanism, eight contracts formerly carrying out these studies have been consolidated under a single management-type contract. The workscope is comprised of four tasks as follows:

Task I	Full Protocol Studies
Task II	High Priority Toxicity Studies
Task III	Specific Organ Testing
Task IV	Automation of Toxicity Data

BATTELLE MEMORIAL INSTITUTE (N01-CM-57005) (FORMERLY N01-CM-12182)

Battelle Columbus Laboratories conducts in vivo testing of new synthetic materials and natural products, of both plant and animal origin, in addition to the secondary evaluation of active materials of interest to the Drug Research and Development program. This testing is conducted at a level of effort equivalent to approximately 50,000 leukemia L1210 tests per annum. Testing is conducted according to current Drug Research and Development protocols, i.e., new synthetic materials are tested in leukemia L1210 and new natural products are tested in the P388 leukemia. If active in the P388 test system, the confirming sample is tested in leukemia L1210. When requested, Battelle Columbus schedules the secondary evaluation of materials of Drug Research and Development interest in the B16 melanoma and Lewis lung carcinoma. Schedule dependency studies are also conducted with materials of potential clinical interest. Tumors are maintained in a tumor bank and are available to the Drug Research and Development program. The Project Officer, dependent upon the current needs of the Drug Research and Development program, may change the tumor assay systems. When requested by the Project Officer, methodology and/or research is conducted.

BELLAIRE ACRES (NIH-74-C-901) (FORMERLY NIH-73-C-832)

This contract furnishes approximately 364,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished

BELLAIRE ACRES (NIH-74-C-901) (FORMERLY NIH-73-C-832) (CONTINUED)

from genetic centers and/or rodent production colonies. This contract terminates May 1975.

BELLAIRE ACRES (NIH-74-C-900) (FORMERLY NIH-73-C-805)

This contract furnishes approximately 78,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers. This contract terminates May 1975.

BEN VENUE LABORATORIES, INC. (N01-CM-53811)

This contract is designed to provide a pharmaceutical facility for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile parenteral products. Specifically, the Contractor performs the following duties: (1) formulation development of sterile parenteral products, (2) production and quality assurance testing of parenteral dosage forms for clinical use, (3) stability surveillance of all dosage forms produced, and (4) preparation of manufacture records on all products produced. All projects are initiated by the Project Officer.

BOWMAN GRAY SCHOOL OF MEDICINE (N01-CM-80950)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

BRIGHAM YOUNG UNIVERSITY (N01-CM-43790)

The major emphasis of this project is the detailed evaluation of selected, new antitumor drugs on folic acid metabolism. In addition to a study of drug action on thymidylate synthetase and dihydrofolate reductase, drug effects on serine transhydroxymethylase, N^5, N^{10}-methylene THF reductase, N^5-methyl THF homocysteine transmethylase, N^5, N^{10}-methylene THF dehydrogenase, N^5, N^{10}-methenyl THF cyclohydrase, 5-amino,4-imidazolecarboxamide ribonucleotide transformylase and glycinamide ribonucleotide transformylase will be studied.

BRISTOL-MYERS COMPANY (N01-CM-33751)

This is a no-cost contract concerned with the marketing of clinically useful nitrosoureas, specifically BCNU, CCNU and if the data warrant, methyl-CCNU. The Contractor summarizes the clinical and other data, prepares and files the New Drug Application (NDA) with the Food and Drug Administration, and continues to prosecute the NDA until it is approved. Once the NDA is approved, the Contractor will arrange for appropriate marketing and other aspects of assuring supplies of the drugs to the public.

BRISTOL-MYERS COMPANY (N01-CM-43759)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxic or marginal activity problems into worthwhile agents. A multitude of unique organisms are isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM-23223)

The major objective of this contract is to develop a general and versatile synthesis of the highly complex and clinically effective dimeric vinca alkaloids, vincristine and vinblastine. The synthesis of some related analogs and derivatives and the preparation of radiolabeled vincristine is being undertaken. In addition, the contract provides for the final isolation, purification and identification of metabolites obtained from samples collected by investigators specified by the National Cancer Institute after in vivo administration of the radiolabeled vincristine.

CALIFORNIA, UNIVERSITY OF (N01-CM-02092)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. To date a total of 3,193 typings have been performed. All typings were done and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda were consistently available through the computer terminal by the end of the next day. A total of 590 sera was tested against a panel of 100 standard cells for which HL-A specificities were known during the past contract year.

CALIFORNIA, UNIVERSITY OF (N01-CM-43706)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in

CALIFORNIA, UNIVERSITY OF (NO1-CM-43706) (CONTINUED)

accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CALIFORNIA, UNIVERSITY OF (NO1-CM-43791)

The goal of this project is to develop and use assays which are capable of detecting and evaluating antitumor agents which can increase the lethal effects of ionizing radiation *in vitro* and *in vivo*. Such effects will be evaluated by cytocidal activity in cell culture, by effects on the cell's ability to repair x-ray-induced DNA damage and by effects on the growth of locally implanted Lewis lung carcinoma *in vivo*.

CALIFORNIA, UNIVERSITY OF (NO1-CM-43798)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

CAPETOWN, UNIVERSITY OF (NO1-CM-33738)

This contract was designed to conduct studies of intensive multidisciplinary therapy of patients with bronchogenic carcinoma and to determine the efficacy of each of a number of therapeutic approaches, possible relationship to morphologic type and clinical stage of the disease, and the influence of concomitant pathology. It was initiated because of important therapeutic leads which might prolong longevity of patients with lung cancer as the most commonly lethal malignant tumor in the United States as well as many other countries.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (NO1-CM-23703)

This contract was initiated to provide an experimental model for human breast cancer therapy. The assay system utilized is the CD_8F_1 spontaneous and first-generation transplanted tumor. During this first contract year, drugs have been tested against the first-generation transplanted tumor. The drugs being tested are those specified by the Division of Cancer Treatment staff and are those with established clinical activity and candidates for clinical trial. The contract is designed to provide a human tumor model which will enable us to: (1) test drugs to determine those with specificity against breast carcinoma, (2) study the influence of treatment schedule on "slow-growing" tumors, and (3) study drug combinations as well as drugs combined with surgery and immunotherapy.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by Drug Research and Development, and fractionates the active extracts. An active synthesis program for novel nitrogen heterocyclic compounds, excluding analogs of natural products, is a component of this contract. Excellent instrumentation is provided.

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CM-12343)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. This contract terminates June 1975.

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CM-53812) (FORMERLY NO1-CM-22004)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artifically contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. Task 1 is concerned with the production of germ-free rodents, counterparts with defined microbial flora and large-scale production; Task 2 in Calco, Italy, produces rodents in bio-containment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CM-53848) (FORMERLY NO1-CM-02043)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-74-C-872) (FORMERLY NIH-73-C-807)

This procurement contract is designed to furnish 78,000 six-week-old CD2F$_1$ (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for Drug Research and Development contract studies. The breeding animals originate from the genetic center at this site.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-75-C-21) (FORMERLY NIH-73-C-839)

This contract furnishes approximately 210,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. This contract terminates May 1975.

CHEMICAL ABSTRACTS SERVICE (NO1-CM-43722)

The Chemical Abstracts Service has participated in installing and is operating the new Chemical Information System in support of the Drug Research and Development accessioning and screening program. This system provides the improved capability to: (1) identify which accessions are new to the Drug Research and Development program and which duplicate or are closely related to known active compounds: (2) produce computer-generated shipping documents and other communications (including structural diagrams) to suppliers, screeners, in-house staff, and the acquisition and storage and distribution contractors who are involved in the accessioning and screening process; (3) maintain inventory control; (4) maintain management and operational control of the accessioning process; and (5) search the files on-line to secure an immediate answer for a possible duplicate sample, analogs of a compound of interest or management inquiry.

CHEMICAL ABSTRACTS SERVICE (NO1-CM-51021)

This contract was a terminal extension to allow an orderly transition to a new Chemical Information System operated under contract NO1-CM-43722. During the life of this contract, beginning in 1965, the entire Drug Research and Development file of structures and names were converted into machine-processable form, name and formula indexes were produced, the first computer checking for duplicates was initiated, and computer analog searching was begun. This contract expired November 1974.

CHESTER BEATTY RESEARCH INSTITUTE (NO1-CM-43736)

This is a service contract for the procurement of unique chemicals and drugs from Great Britain and to establish a joint testing program with the Chester Beatty Research Institute. The Contractor will perform all liaison activities required to procure unique samples for the National Cancer Institute screening

CHESTER BEATTY RESEARCH INSTITUTE (N01-CM-43736) (CONTINUED)

program. The Contractor will: (1) further develop a model tumor system, including human carcinomas of the breast, lung, colon and ovary, grown in CBA or other mice specifically deprived of T-lymphocytes by thymectomy, x-irradiation, and hematopoietic therapy with syngeneic bone marrow, (a) examine various methods of transplantation, (b) investigate various methods of assessing antitumor effectiveness, (c) establish a method of comparing the response of the tumor in the mouse with the response obtained in patients receiving chemotherapy, based upon clinical evaluation of known antitumor agents; (2) further develop a model tumor system for carcinoma of the human breast growing in nude mice. The points for investigation are the same as those described in relation to immuno-incompetent CBA mice referred to under 1 above; (3) determine which of a range of commonly used chemotherapeutic agents is effective against screening tumors growing in deprived mice.

CHICAGO, UNIVERSITY OF (N01-CM-33748)

This contract is intended to support studies to isolate and compare the dihydrofolate reductase from normal and malignant tissues, primarily solid tumors, in order to determine any detectable molecular differences in these enzymes. The study also utilizes *in vitro* testing to help design inhibitors of human and subhuman dihydrofolate reductase. These are aimed at understanding the mechanism of resistance developed in human malignancy to antifols.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM-43803)

This contract was designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Two drugs, Diglycoaldehyde, NSC-118994, and Dianhydrogalactitol, NSC-132313, have been studied in children with acute leukemia and solid tumors.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM-53831)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of stages III and IV neuroblastoma, rhabdomyosarcoma and other small-cell sarcomas. Trials were recently begun and three patients have been put on study.

CLEVELAND CLINIC (N01-CM-33704)

This contract provides support for a protocol study in which patients with severe psoriasis will be randomly allocated to therapy with either methotrexate (MTX) on two different schedules or a control drug. Evaluation of hepatic function will be performed at regular intervals and data will be collected to determine whether hepatotoxicity in psoriasis is due to MTX or is coincidental.

COLLABORATIVE RESEARCH, INC. (NO1-CM-12326)

The objective of this contract is the design and synthesis of oligo- and polynucleotides as potential inhibitors of RNA-dependent DNA polymerase and other DNA polymerases. It is anticipated that the synthetic nucleotides may act as templates and primers for different enzymes and may inhibit viral and leukemic DNA polymerases specifically without interfering with the normal nucleic acid metabolism of the host cells.

COLLABORATIVE RESEARCH, INC. (NO1-CM-60506)

The major objective of this contract is the synthesis of some potential metabolites of cyclophosphamide and phosphoramide mustards as latent cytotoxic agents. In addition, the preparation of some analogs and derivatives of cyclophosphamide is being undertaken.

COLLEGE OF MEDICINE AND DENTISTRY OF NEW JERSEY (NO1-CM-43760) (FORMERLY NO1-CM-23211)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

COLORADO STATE UNIVERSITY (NO1-CM-12192)

The objective of this contract is to investigate the pathogenesis of Adriamycin/Daunomycin-induced cardiotoxicity utilizing clinical, morphological and biochemical parameters. The rabbit appears to be the most promising species for assessing the cardiotoxicity of this class of anticancer agents. Studies have been designed to establish optimal dose and time schedules to provide the highest incidence and maximum consistency of cardiotoxicity. Adriamycin analogs are being screened for cardiotoxicity using the rabbit with the hope of finding one with a maximum therapeutic effect and minimal cardiotoxicity. The rabbit is also being used for antidotal studies.

COLORADO, UNIVERSITY OF (NO1-CM-12186)

Tumor tissues have consistently been shown to contain increased amounts of the enzymes which methylate pre-formed transfer RNA, the tRNA methylases. The Contractor has found naturally occurring inhibitors of the tRNA methylases in mature organ tissue, which are either lacking or inactive in malignant tissues. The purposes of this contract, then, are threefold, as follows:

1. To study the distribution and alterations of these inhibitors in tissues undergoing malignant transformation.
2. To further define the exact biochemical role the inhibitors have in the regulation of cellular differentiation and growth.
3. To determine if these inhibitors may be useful therapeutic agents for the treatment of tumors.

COLORADO, UNIVERSITY OF (NO1-CM-43779)

This contract is for the chemical synthesis of a number of new and novel nitrogen heterocyclic compounds as potential anticancer agents. The major emphasis is on preparing compounds containing the triazepine and oxathiazine ring systems. All materials prepared, including intermediates, are submitted in adequate quantities for antitumor testing.

COMMUNITY BLOOD AND PLASMA SERVICE (NO1-CM-42150)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets primarily to the leukemia service of the National Cancer Institute. The use of HL-A matched platelets decreases the risk of sensitization or production of antibodies to white cells and platelets, therefore, provides efficient and effective platelet support therapy. During the first eight months of FY-1975, 2,112 donors were processed.

CONNECTICUT, UNIVERSITY OF (NO1-CM-12181)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CONTROL DATA CORPORATION (NO1-CM-33739)

The purpose of this contract is to provide data processing and computer support which enables simple, rapid and on-demand retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols.

DOW CHEMICAL COMPANY (NO1-CM-12165)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

DOW CHEMICAL COMPANY (NO1-CM-23712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: (1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; (2) Phase II, teratology studies using mice and/or rats and rabbits; and (3) Phase III, perinatal studies employing mice and/or rats.

DOW CHEMICAL COMPANY (NO1-CM-33724)

It has been demonstrated that some derivatives of rifamycin have significant inhibitory activity for viral and cellular DNA polymerases (including reverse transcriptase). The work of this contract is designed to synthesize rifamycin derivatives which are more effective inhibitors of RNA-dependent DNA polymerases. Additionally, the development of a strong selective inhibitor of RNA-dependent DNA polymerases could help in clarifying the mechanism of action of this enzyme and possibly lead to new cancer chemotherapy agents through chemical modifications of 3-formylrifamycin-SV.

DUKE UNIVERSITY (NO1-CM-02030)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

DUKE UNIVERSITY (NO1-CM-22044)

The major objectives of this contract are (1) the development of measures of sensitivity and quantity in autoradiography, and (2) the improvement of sensitivity and resolution of the autoradiographic process. Because of loss of key personnel at Duke University, completion of data acquisition phase of this contract was delayed. Accordingly, the contract was extended for an additional six months without additional funds in order to permit Duke personnel to complete this phase, employing the autoradiographic grain counter developed at the National Cancer Institute. Data analysis has begun on material so developed and will continue so as to be completed on or about June 1975.

EMORY UNIVERSITY (NO1-CM-43764)

This contract provides support to perform Phase II studies of the response rate of hepatocellular carcinoma, cholangiocellular carcinoma, gallbladder carcinoma and metastatic bioduct carcinoma to adriamycin and other drugs; to correlate response of alpha-feto protein and human chorionic gonadotropin titers with clinical antitumor response; and compare response rate of these diseases in Zambian Bantu patients with that of United States patients.

FLOW LABORATORIES, INC. (NIH-74-C-894) (FORMERLY NIH-73-C-838)

This contract furnishes approximately 215,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

FRANKLIN INSTITUTE (NO1-CM-53771)

This contract provides for the preparation of *Cancer Therapy Abstracts*, Volumes 16 and 17 for the years 1975 and 1976. The journal is supplied as hard copy and in computer-readable form. This current awareness tool contains abstracts of the world's literature pertinent to cancer therapy.

GEORGE WASHINGTON UNIVERSITY (NO1-CM-12206)

This Contractor is continuing studies on the effects of drug combinations on biochemical parameters in the canine spontaneous lymphosarcoma and certain normal tissues. These investigations are attempting to determine whether certain drugs in combination with methotrexate will alter this antifol's permeability and biochemical effect.

GEORGETOWN UNIVERSITY (NO1-CM-12123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to

GEORGETOWN UNIVERSITY (NO1-CM-12123) (CONTINUED)

make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGETOWN UNIVERSITY (NO1-CM-33745)

The Eastern Cooperative Oncology Group consists of 350 members and 35 cooperative institutions plus subsidiary hospitals. The Operations Office, funded by this contract, provides executive and administrative support for activities of the Group. On request from investigators, provides randomization and Group correspondence; obtains data from the investigators and maintains liaison with appropriate members of the National Cancer Institute staff. The Contractor has continued to function well during the past contract year and has been responsible for changing the activities of the ECOG along more disease-oriented lines.

GEORGETOWN UNIVERSITY (NO1-CM-43732)

This contract has as its main objective the production of novel actinomycins by using biotransformation and co-metabolism fermentation techniques. These novel actinomycins will be produced in pure form in 100-500 mg. quantities and made available for broad-tumor screening. The use of many different compounds as substrates will be thoroughly explored.

HARLAN INDUSTRIES, INC. (NO1-CM-23218)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments. This contract terminates April 1975.

HARLAN INDUSTRIES, INC. (NIH-74-C-931) (FORMERLY NIH-73-C-844)

This contract furnishes approximately 78,000 six-week-old CD2F$_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARLAN INDUSTRIES, INC. (NIH-74-C-932) (FORMERLY NIH-73-C-845)

This contract furnishes approximately 260,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARVARD UNIVERSITY (NO1-CM-53825)

This project is based upon the exploration of the possibility that disarming malignant cells by increasing their immunogenicity by altering their cell surface polysaccharide composition, may be an effective chemotherapeutic approach. A detailed study of the effects of naturally occurring sugars and of sugar analogs on the oncogenicity of tumor cells and on the composition of their membrane glycoproteins, is being performed.

HAWAII, UNIVERSITY OF (NO1-CM-33747)

This contract provides for the collection of plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are reproducibly active in the Drug Research and Development tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by Drug Research and Development.

HAZLETON LABORATORIES, INC. (NO1-CM-23701)

Mixed leukocyte cultures are an important determinant of the degree of compatibility of two individuals relative to transplantation antigens. The first objective of this contract is the selection of MLC and compatible donor recipient pairs for use in an active canine and human marrow grafting and transfusion program; second objective is the continued improvement of the MLC method for use with smaller quantities of patient blood and greater reproducibility; and third objective is the continued immune evaluation of patients on chemotherapy. In addition, a large liquid nitrogen freezer for storage of blood and bone marrow samples will be purchased.

HAZLETON LABORATORIES, INC. (NO1-CM-33705)

This contract provides for microbiological services in support of the National Cancer Institute, Division of Cancer Treatment, clinical investigations at the Baltimore Cancer Research Center, and for special testing pertinent to this program. The services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators, as well as final summarized microbiological reports for patients' medical records.

HAZLETON LABORATORIES, INC. (NO1-CM-33708)

The major objectives of this contract are: (a) to obtain model tumor systems and pre-neoplastic changes in primates in order to evaluate the potential usefulness of various anticancer agents and immune stimulants for man; (b) to evaluate the long-term effects of antineoplastic agents which are being used clinically for long-term remissions and in the treatment of diffuse collagen disorders; (c) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (d) to develop biological markers and diagnostic tests for detecting pre-neoplastic changes as well as frank neoplasia; (e) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical and immunological studies; and (f) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, National Cancer Institute.

HAZLETON LABORATORIES, INC. (NO1-CM-43702)

This contract provides for the supply of dogs for leukocyte transfusion and transplantation experiments. Contractor supplies animals with spontaneous neoplasms in order to evaluate the immunotherapeutic effectiveness of bone marrow transplantation and immunotherapeutic manipulation following chemotherapy regimens.

HAZLETON LABORATORIES, INC. (NO1-CM-43705)

The contract performs studies to determine the pharmacologic and toxicologic parameters of antineoplastic agents which are administered intrathecally. Appropriate drugs of either routine preparation or special formulation are provided by the National Cancer Institute, Drug Research and Development. The exact treatment schedules and experimental details are determined by the Project Officer in coordination with the Principal Investigator. Every effort is made to keep protocols consistent so that comparison between drugs may be made.

HAZLETON LABORATORIES, INC. (NO1-CM-57007) (FORMERLY NO1-CM-23704)

This contract provides *in vivo* screening of new test materials, both natural products and synthetic, and the subsequent testing of active materials. The current testing level is at a rate of approximately 25,000 tests per year. The P388 leukemia is routinely used for the initial testing of natural products. Those demonstrating efficacy are also tested in L1210. The L1210 leukemia is routinely used for all new synthetic materials. Limited testing is conducted against the intracerebrally implanted ependymoblastoma. Treatment schedule studies as well as research and development work is conducted as requested by the Drug Evaluation Branch. The Drug Research and Development protocols are followed for all testing.

HAZLETON RESEARCH ANIMALS, INC. (NIH-74-C-1150) (FORMERLY NIH-73-C-879)

This contract provides 268 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HEBREW UNIVERSITY (HADASSAH MEDICAL SCHOOL) (NO1-CM-12127)

This research contract is concerned with the effects of an extract, MER (methanol extractable residue), obtained from attenuated tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity.

HERNER INFORMATION SERVICES, INC. (NO1-CM-23244)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts (Volumes 13 and 14 for 1972 and 1973). This contract expired December 1974.

HERNER INFORMATION SERVICES, INC. (NO1-CM-43720)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts (Volume 15 for 1974). Computer tapes of the abstracts and indexes are also supplied. The price of the annual subscriptions pays the cost of printing and mailing by the Contractor. This current awareness journal, published monthly, supplies abstracts and annotations of the world's literature pertinent to the treatment of cancer.

HORTON LABORATORIES, INC. (NIH-74-C-902) (FORMERLY NIH-73-C-837)

This contract furnishes approximately 172,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

IIT RESEARCH INSTITUTE (NO1-CM-43755)

This represents a support services contract to provide assistance to the staff in preliminary data evaluation and follow-up on materials found active in the presumptive screen. The Contractor participates with staff in monitoring testing and in expediting work on those materials which appear to have the greatest promise, based upon initial activity.

IIT RESEARCH INSTITUTE (NO1-CM-53829)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella* spp. and *Pseudomonas* spp. in program rodents and

IIT RESEARCH INSTITUTE (N01-CM-53829) (CONTINUED)

other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. The scope of performance of this contract was changed recently to the extent that they are now monitoring animals leaving the genetic and rodent production centers primarily for the presence of ecto- and endoparasites. They also forward blood serum samples to the College of Medicine and Dentistry of New Jersey where these same animals are monitored for virus diseases. The results of these monitoring studies will be utilized to upgrade the quality of animals being sent to the laboratories. Diagnostic procedures are also carried out at this facility when disease problems occur in the various animal breeding colonies or in the laboratories using these animals.

IIT RESEARCH INSTITUTE (N01-CM-57004) (FORMERLY N01-CM-81021)

Thie contract provides for the performance of in vivo testing of new synthetic materials and natural products. Screening is being conducted according to Drug Research and Development protocol at a level of approximately 50,000 tests per year. New natural products are being tested in the P388 leukemia in the mouse host. The lymphoid leukemia L1210 tumor test system is utilized for all synthetics. Testing is conducted in the B16 melanoma and Lewis lung carcinoma tumor systems when requested by DR&D staff. Statistical analysis and characterization studies of the first generation AKR mouse leukemia test system have been completed this year. These studies determined the correlation with the spontaneous AKR test system and the feasibility of both single drug and drug combination treatments in the first generation test system. Work is beginning with a murine renal carcinoma as a potential organ-targeted system. All methodology and development is carried out only at the request of the Project Officer and during this year has been devoted to the development of new in vivo screening tumor test systems.

ILLINOIS, UNIVERSITY OF (N01-CM-22078)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

ILLINOIS, UNIVERSITY OF (N01-CM-23208)

The principal objectives of this contract are as follows: (1) to provide the Division of Cancer Treatment, National Cancer Institute, with samples (not previously available) of a number of ansamycin-type antibiotics for biological and related testing. This group includes the streptovaricins, tolypomycins and geldanamycins, at least some of which are highly potent RNA-dependent DNA polymerase inhibitors and also have antiviral activity. Thus, detailed study of as many members of this group as possible is considered imperative; and (2) to isolate several presently unidentified streptovaricin components in quantity sufficient for characterization and biological evaluation.

INDIAN CANCER RESEARCH CENTRE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing *in vivo* of these extracts as well as synthetic materials synthesized in India.

For this purpose, an *in vivo* screening laboratory is maintained in Bombay, India together with an animal breeding colony to supply the necessary host animals. Currently, the L1210 lymphocytic leukemia and the P388 lymphoid leukemia are utilized with testing conducted as per current Drug Research and Development protocols. The L1210 tumor system is employed for the testing of synthetic materials with the P388 tumor system employed for the testing of natural products. Those active in this system are tested in the L1210 tumor system.

INDIANA UNIVERSITY FOUNDATION (N01-CM-02028)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

INSTITUT JULES BORDET (N01-CM-53840)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. In addition to the information activities, a screening program is maintained for the testing of selected new compounds, in accordance with National Cancer Institute protocols.

INSTITUTE OF CANCER RESEARCH, ROYAL CANCER HOSPITAL (N01-CM-23717)

The objectives of this contract are to study the effects of chemotherapeutic agents in experimental solid tumor systems and in human xenografts transplanted into immune-suppressed mice in relation to their proliferative state and their vascular supply, and compare the effects of these agents on the hematopoietic system. In the past, quantitative *in vivo* and *in vitro* clonogenic assays were developed for B16 melanoma and Lewis lung carcinoma and for normal marrow. More recently, *in vivo* diffusion chamber assays have been developed which will permit comparisons of clonogenic cell populations in mouse and human normal and tumor tissues in the same experimental system. Interrelationships among the effects of nitrosoureas, alkylating agents and radiation have been studied.

Studies are in progress comparing cell survival in tumor and marrow following other drugs in single and multiple doses and in two-drug combinations. Radiotracer methods have been developed to measure tumor blood supply. These methods will be used to study proliferative and drug response characteristics in tumors in relation to their blood supply.

IOWA, UNIVERSITY OF (NO1-CM-43743)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for clinical evaluation. This Contractor also has the capability to produce products for oral use if required. The Contractor is responsible for completing required analytical and safety tests on each product prepared. All work is assigned and reviewed by the Project Officer.

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" (NO1-CM-23242)

This Contractor is continuing studies aimed at refining techniques both for measuring antineoplastic drugs in biological fluids and the effects of these drugs on measurable biological parameters in host's tumor as well as normal tissues. Comparative immunodepressive activities of adriamycin and daunomycin are being investigated. Increased concentration of adriamycin in hearts of tumor-bearing mice is being further investigated.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM-33714)

The purpose of this contract is the evaluation of new anticancer drugs alone or in combination with conventional agents in prospective controlled clinical trials. Four studies are being performed: (1) adjuvant combination chemotherapy for breast cancer with positive axillary nodes; (2) controlled study with multiple drug combinations for metastatic breast cancer; (3) controlled study of new chemotherapeutic regimens in advanced carcinoma of stomach, colon and rectum; and (4) a controlled study of BCNU, CCNU and irradiation in the treatment of malignant glioma.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM-43726)

The purpose of this contract is to support the investigation of protocol studies in metastatic melanoma using dibromodulcitol, streptozotocin and imidazole carboxamide, and a surgical adjuvant study of a controlled trial of prolonged chemotherapy, immunotherapy or a combination of both modalities.

JACKSON LABORATORY (NIH-75-C-3) (FORMERLY NIH-74-C-125)

This service contract provides for the procurememt of approximately 62,400 C57BL/6 and 10,400 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 78,000 AKR inbred mice for compound evaluation studies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM-22054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. A small testing facility is also maintained for the screening of selected new compounds.

JEFFERSON MEDICAL COLLEGE (NO1-CM-02136)

The purpose of this contract is to obtain information on the disposition and metabolism of new agents entering Phase I clinical trial. A number of agents, including dibromodulcitol, 5-azacytidine, streptozotocin and Yoshi 864, have been studied under this contract. Work is currently being completed on the latter compound using radiolabeled material to determine its metabolism and disposition. This contract has been recompeted in FY-1976, and the final decision is pending.

JOHNS HOPKINS UNIVERSITY (NO1-CM-43718)

The synthetic and catabolic rates of specific myeloma paraprotein will be studied utilizing radioactive guanidoarginine for labeling purposes in mice bearing a transplanted myeloma cell line. Efforts will be made to correlate total cell number with the above parameters in an attempt to work out a possible method for determining this number. Comparison will be made to an existing system which requires culture of the involved cells to determine synthetic rate.

KANSAS, UNIVERSITY OF (NO1-CM-12184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Methotrexate, 5-fluorouracil and melphalan are currently under study.

KANSAS, UNIVERSITY OF (NO1-CM-23217)

This contract provides research capabilities in the area of formulation design and development. The research involves the development of intravenous dosage

KANSAS, UNIVERSITY OF (N01-CM-23217) (CONTINUED)

forms of new drug substances having formulation problems. A second objective of this research contract is to develop new and unique delivery systems for poorly manageable drugs. The drugs to be researched are assigned by the National Cancer Institute staff through the Project Officer.

KANSAS, UNIVERSITY OF (N01-CM-43802)

This contract was designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute, or to conduct Phase I clinical studies with new combinations or regimens mutually agreed. Protocols on Phase I studies with dianhydrogalactitol, NSC-132313, Anguidine, NSC-141537, and parenteral MeCCNU, NSC-95441, have been approved.

KANSAS, UNIVERSITY OF (N01-CM-53819) (FORMERLY N01-CM-12010)

This Contractor maintains a genetic production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in Drug Research and Development program investigations. Its spectrum includes three strains: C57BL/6, DBA/2 and BSVR/Sr. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies. Hybrids for special studies are produced as required for specific studies.

KENTUCKY, UNIVERSITY OF (N01-CM-71173)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

LABORATORY SUPPLY COMPANY, INC. (N01-CM-02041)

This Contractor maintained a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony were made available for tumor transplantation and the majority were furnished to large-scale production colonies. This contract terminated November 1974.

LABORATORY SUPPLY COMPANY, INC. (N01-CM-53775)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing

LABORATORY SUPPLY COMPANY, INC. (N01-CM-53775) (CONTINUED)

laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments.

LABORATORY SUPPLY COMPANY, INC. (NIH-74-C-892) (FORMERLY NIH-73-C-840)

This contract furnishes approximately 260,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (NIH-74-C-893) (FORMERLY NIH-73-C-835)

This contract furnishes approximately 78,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LEEDS, UNIVERSITY OF (N01-CM-43727)

The purpose of this contract is the investigation of the usefulness of various "markers" in the study of gastrointestinal cancers with special reference to the earlier detection of metastases and their pattern of change during the evolution of the disease.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CM-53814) (FORMERLY N01-CM-33729)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artifically contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which, in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (N01-CM-12340)

The objectives of this project are (1) to purify viral antigens from RNA tumor viruses and human leukemic cells and to prepare antisera against these antigens, (2) to develop diagnostic and prognostic tools for human leukemia, and (3) to expand basic research efforts in tumor cell biochemistry in general in order to achieve a better understanding of the neoplastic process and its prevention and treatment.

LITTON BIONETICS, INC. (NO1-CM-22062)

The main objective of this contract is the preparation, growth and characterization of defined tissue culture cell lines and of fresh human leukemic blood cells for use by Project Officers. Cell cloning, cell genetics, cytochemistry, defining immunological cell markers and labeling cells with radioisotopic precursors for the isolation of metabolic products are also performed.

LITTON BIONETICS, INC. (NO1-CO-25423)

At the Frederick Cancer Research Center, the Division of Cancer Treatment is developing a fermentation pilot plant for the large-scale production and isolation of microbial products of interest to the program. Facilities will also be available for the production of other natural products. A fermentation research laboratory has been completed and will be used to systematically evaluate various microbes' abilities to ferment unique substrates and produce novel compounds. The Division also participates in the operation of the Animal Farm and environmental control aspects of the program at FCRC.

LITTON BIONETICS, INC. (NO1-CM-33741)

This contract provides a source for the screening of selected drugs for inhibitory activity for selected mammalian cell and viral nucleotide polymerase enzymes (*in vitro*) and for toxicity or specific inhibition of oncogenic viral replication and transforming capacity in cell culture systems. Enzymes employed are the Simian sarcoma virus reverse transcriptase, mammalian DNA polymerases I and II, RNA polymerases I and II and Poly-A polymerase. Cell culture effects on DNA, RNA and protein synthesis, cell proliferation and murine sarcoma virus focus formation in 3T3 cells are measured. The screening operation includes a preliminary cell culture and anti-enzymatic screen for all compounds and a secondary cell culture and anti-enzymatic evaluation for compounds demonstrating significant activity in the primary screen. Materials submitted to these screens are selected by the Project Officer.

LITTON BIONETICS, INC. (NO1-CM-53778)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella spp*. and *Pseudomonas spp*. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples will be screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques.

MADRID, UNIVERSITY OF (NO1-CM-53792)

The object of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems

MADRID, UNIVERSITY OF (N01-CM-53792) (CONTINUED)

used are isolated heart and liver mitochondria, and intact Ehrlich ascites cells, using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores. In addition, the action of selected drugs on myocardial membrane Na-K dependent ATPase is being evaluated.

MAKERERE UNIVERSITY COLLEGE (N01-CM-71343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to provide facilities for direct collaborative studies to the members of the National Cancer Institute, including Medical Oncology area, Laboratory of Pharmacology, Immunology, Pathology and Etiology. Research is continuing as previously under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny.

"MARIO NEGRI" INSTITUTE FOR PHARMACOLOGICAL RESEARCH (N01-CM-33720)

The main purpose of this project is to establish a regional European contract as an extension of the Division of Cancer Treatment compound acquisition and screening program. The Contractor will collect approximately 1,500 materials in Southern European countries and perform primary screening at a rate of approximately 7,500 L1210-equivalency tests per year.

MARSHALL RESEARCH ANIMALS, INC. (NIH-75-C-10) (FORMERLY NIH-73-C-889)

This contract provides 360 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (N01-CM-43748)

Under this service contract, the University of Maryland at Baltimore will provide suitable facilities, administrative support, ancillary support services and personnel for the inpatient operation of the Baltimore Cancer Research Center, as well as space for activities such as outpatient department, cell component therapy, patient care pharmacy and laboratory efforts necessary for the conduct of the program.

MASON RESEARCH INSTITUTE (N01-CM-57003) (FORMERLY N01-CM-12259)

This service contract is for the in vivo testing of synthetic and natural products which are submitted to Drug Research and Development. The L1210 tumor system is utilized for the testing of synthetic materials with natural products tested initially in the P388 leukemia with those demonstrating efficacy being tested in the L1210 leukemia. Testing is proceeding at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine the optimal dose, schedule and route of administration, combination studies and special studies on other tumor test systems are conducted as directed by Drug Research and Development staff.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CM-43712)

The objectives of this project are (1) the unique conceptual division of the bleomycin molecule into five complex but synthetically obtainable intermediates, (2) the synthesis of each of the unique parts for biological testing and comparison with the analogous part from the natural product where possible, (3) the determination of the correct stereochemistry for those six centers still unknown, and (4) the joining of the five parts, one at a time in the proper sequence, in order to determine whether all five parts are necessary for antitumor activity. The Contractor will submit samples of intermediates as well as final products for in vivo and in vitro evaluation by the National Cancer Institute. These compounds will be fully characterized as to purity and structure.

MAYO FOUNDATION (N01-CM-02066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment Program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at the National Cancer Institute. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (N01-CM-12185)

This contract was designed to provide information on patients on the effect of chemotherapy in the growth and size of breast cancer lesions and toxicity. Selection of drugs and protocol were mutually agreeable to the Project Officer and the Mayo Foundation. This contract expired September 1974.

MAYO FOUNDATION (N01-CM-33735)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

MAYO FOUNDATION (NO1-CM-43715)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

MAYO FOUNDATION (NO1-CM-43783)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

MAYO FOUNDATION (NO1-CM-43796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MAYO FOUNDATION (NO1-CM-53772)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

MAYO FOUNDATION (NO1-CM-53838)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed. Dianhydrogalactitol, NSC-132313, has been studied in adult patients with solid tumors.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM-43716)

This contract was designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed. Piperazinedione, NSC-135758, has been studied in solid tumors in adults and children.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM-43804)

This contract has been designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National

Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

MERCK AND COMPANY (NO1-CM-22002)

This contract provides for the resynthesis of compounds in the amounts needed for clinical trial or for necessary preclinical evaluations. About 25% of the budget is devoted to clinical preparations. Because of their extensive and diverse experience, the Contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which cannot be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (NO1-CM-02225)

This contract provides for *in vitro* screening of synthetics and natural products and follow-up testing of natural products having demonstrated correlative activity in the Drug Research and Development *in vivo* screens, as a tool for fractionation. Procedures are those described by DR&D protocols and methodology is carried out only at the request of the Project Officer. Approximately 23,000 tests at three to five dose levels per test are performed yearly.

MIAMI, UNIVERSITY OF (NO1-CM-43800)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MICHIGAN CANCER FOUNDATION (NO1-CM-53789)

The objective of this project is to characterize the range of molecular biological effects of antitumor drugs. In addition to effects on nucleic acid synthesis, the effects of new drugs on DNA structure, cell membrane permeability, cell surface electrical charge, cell surface adhesive properties, and enzymes involved in the synthesis of cell surface glycoproteins will be studied.

MICHIGAN DEPARTMENT OF HEALTH (NO1-CM-33702)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program services by this assay contract is financed by the Michigan Department of Health.

MICHIGAN, UNIVERSITY OF (N01-CM-92004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM-33728)

This contract is addressed to the detailed investigation of problems which arise in the chemotherapy of malignant disease. The effort is divided into basic divisions, all of which have been competed during this reporting period.

Chemotherapy Research - Activities in this division are addressed to the exploration of new and potentially useful methodology for prediction, evaluation and improvement of drug therapy; the investigation of novel schedules of treatment; the investigation and application of predictability models for combination chemotherapy; and studies related to remission induction and maintenance with direct reference to problems and findings in clinical chemotherapy. This segment of the effort will be terminated in June 1975.

Drug Evaluation - The drug evaluation and biochemical pharmacology (drug metabolism) tasks emphasize the rapid screening of synthesis contractor analogs in in vivo assays designed specifically on the basis of the characteristics of the parent active agent; on the detailed evaluation of "active" drugs; on the evaluation of combinations of drugs of immediate clinical interest; and the study of the physiological disposition and metabolism of antitumor drugs and drug interactions, with special attention to therapeutic effectiveness and toxicity. These studies have been discontinued under this contract.

Immunology - The role of this division is to conduct basic and applied research in the area of tumor immunology which may lead to better procedures for controlling or suppressing tumor growth by the use of immunotherapy employed alone or in combination with antineoplastic drugs. This portion of the contract will be terminated in June 1975.

Laboratory Animal Science - The functions of this division are the development of new hybrids of inbred animals and the provision of holding facilities and animal caretaking services for the research studies conducted under the total contract. This type of support was prorated for each portion of the contract requiring such services and has been appropriately incorporated into the new competitive awards.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-33728) (CONTINUED)

The former programs in organic chemistry and drug distribution were competed during Calendar Year 1974 and are being continued under Microbiological Associates, Inc., contracts NO1-CM-43761 and NO1-CM-43793, respectively.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-43761)

The major objective of this contract is the synthesis of new potential antitumor agents. The synthesis is directed at following up active leads from natural and synthetic sources. The areas of investigation include the preparation of analogs and derivatives of sparsomycin, mycophenolic acid, indicine N-oxide, ethidium chloride and quinaldine; and the oxidation products of selected polysaccharides. All materials prepared, including intermediates, are submitted for antitumor testing.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-43793)

The function of this contract is to provide the National Cancer Institute with services for the storage and distribution of bulk drugs and chemicals to various research laboratories throughout the country. The contract also provides for the weighing, packing and shipping of chemical samples and the maintenance of accurate inventory records.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-53818)

This genetic production center supplies a variety of inbred and hybrid rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of five strains of rodents.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CB-92177)

The Contractor performs routine serologic assays for the determination of alloimmunization on National Cancer Institute patients and potential platelet and leukocyte donors. Contractor serves as a serum repository for serialized serum specimens from all patients on the Pediatric Oncology Branch and has continued to perform well.

MIDWEST RESEARCH INSTITUTE (NO1-CM-33722)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for Drug Research and Development. The Contractor determines the identity and purity of these materials, developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromotographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation. This contract provides the necessary analytical data for filing with the Food and Drug Administration.

MIDWEST RESEARCH INSTITUTE (N01-CM-33743)

Midwest Research Institute is one of three major synthetic contractors for Drug Research and Development. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to DR&D for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around the synthesis of (1) potential tRNA O-methyltransferase and protease inhibitors, (2) receptor site-oriented antileukemic compounds based on the N-O-O triangulation hypothesis developed by this Contractor, (3) coralyne, nitidine, bleomycin, streptonigrin, and other natural products and their analogs, and (4) analogs of procarbazine. The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MILES LABORATORIES (N01-CM-43709)

This is a no-cost contract concerned with the marketing of a clinically useful drug, DTIC. The Contractor summarizes the clinical and other data, prepares and files the New Drug Application (NDA) with the Food and Drug Administration, and continues to prosecute the NDA until it is approved. Once the NDA is approved, the Contractor will arrange for appropriate marketing and other aspects of assuring supplies of the drug to the public.

MINNESOTA, UNIVERSITY OF (N01-CM-43788)

The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs are being evaluated.

MISSOURI, UNIVERSITY OF (N01-CM-12323)

The objective of this contract is to develop procedures involving various advanced high resolution analytical techniques using specific instrumentation (e.g., GLC, amino acid analyzer, mass spectrometer, etc.) to measure quantitatively specific and potential biologic markers in the urine and blood of cancer patients and to utilize these procedures to study patients before, during and after therapy. Methods have been established for assay of polyamines (putrescine, spermidine, cadaverine and spermine) and methylated nucleic acid derivatives, as well as other minor nucleic acid bases in urine and clinical studies are in progress to determine the value of these markers for following disease status in patients. In addition, preliminary work has been initiated for the assay of several other potential markers in serum and urine including serum fucose/protein ratio, urinary β-aminoisobutyric acid, and hydroxyproline/creatinine ratio.

MONSANTO RESEARCH CORPORATION (NO1-CM-23715)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

MONSANTO RESEARCH CORPORATION (NO1-CM-33709)

This service preparative contract provides for the large-scale extraction, isolation and purification of natural products and the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM-43703)

The objective of this contract is to identify the antitumor activity of new and old agents against advanced stage II and IV ovarian carcinoma.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM-53837)

This contract has been designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM-53841)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

MURPHY BREEDING LABORATORIES, INC. (NIH-74-C-875) (FORMERLY NIH-73-C-868)

This procurement contract provides for the supply of 200,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

MURPHY BREEDING LABORATORIES, INC. (NIH-74-C-876)

This contract furnishes approximately 52,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (N01-CM-53849) (FORMERLY N01-CM-40044 - TASK ORDER 16)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL ACADEMY OF SCIENCES (N01-CM-53850) (FORMERLY N01-CM-40044 - TASK ORDER 6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by *ad hoc* committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories and non-profit research institutions.

NATIONAL NAVAL MEDICAL CENTER (Y01-CM-50100)

This interagency agreement provides support to the Hematology-Oncology Section of the Bethesda Naval Medical Center. The agreement is designed to integrate the breast cancer studies of the Bethesda Naval Medical Center and the National Cancer Institute's Medical Breast Cancer Section. Joint therapeutic protocols for patients with primary and metastatic disease are in the program. Patient accrual to the studies has been satisfactory.

NAVAL WEAPONS CENTER (Y01-CM-50101)

This project will develop and evaluate prototype instruments for the quantitation of growth in granulocyte precursor cells. The monitoring technique closely follows that developed in missile guidance systems for remote detection

NAVAL WEAPONS CENTER (Y01-CM-50101) (CONTINUED)

of small targets against complex bright backgrounds. The National Cancer Institute and National Heart and Lung Institute laboratories are collaborating in the design and evaluation of three prototype instruments for ongoing Division of Cancer Treatment studies of leukopoietic mechanisms, the assessment of abnormalities in white cell proliferation, and the determination of marrow function associated with other diseases.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (N01-CM-02035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling Drug Research and Development to maintain and distribute tumors identical to the prototype: (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (N01-CM-33726)

The objective of this contract is the development of antiviral-antitumor agents from a class of ansa macrolide compounds, the streptovaricin complex. These agents were first shown by the Principal Investigator to be reverse transcriptase inhibitors and to interfere with viral replication. A significant finding has been their later observation of relatively selective *in vivo* effects, i.e., minimum cytotoxicity yet interference with viral replication. Bioassay studies are being carried out on components of the crude complex isolated by Dr. Rinehart at the University of Illinois; the active component does not appear to be any of the isolated, purified streptovaricins.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (N01-CM-33734)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (N01-CM-43714)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats stage II nonseminomatous

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-43714)
(CONTINUED)

lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-43782)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-43785)

The objective of this project is to develop a new test system, Profile of Drug Interactions. The approach to be used involves a determination in cell culture of the types of interaction (i.e., synergistic, additive or antagonistic) between the new drug and a selected panel of known chemotherapeutic agents or metabolic effectors. The interaction profile pattern produced can then be compared with those of other antitumor drugs to detect similarities in mechanisms of action. This system has successfully identified a group of structurally dissimilar inhibitors of ribonucleotide reductase as having similar biochemical actions.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-43794)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-53834)
(FORMERLY NO1-CM-22072)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM-71174)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

NOTRE DAME, UNIVERSITY OF (N01-CM-23718)

Leukemia patients under treatment by chemotherapy are highly vulnerable to infection. Microorganisms that are part of the normal human flora may themselves cause serious infection. During the course of investigations under this contract, AKR mice have been successfully decontaminated and remained so when maintained under sterile conditions. These mice, when treated with immunosuppressive drugs, survived just as readily as do germ-free animals.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM-33736)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

OHIO STATE UNIVERSITY (N01-CM-71175)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

OREGON STATE UNIVERSITY (N01-CM-23201)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical trials. Specifically, the three nitrosoureas, BCNU, CCNU and MeCCNU, are being studied from two points of view. First, the complicated metabolic alterations these drugs undergo are being studied in detail. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs is under investigation.

PARKE, DAVIS & COMPANY (N01-CM-23708)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 50% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large-scale extraction, isolation and purification of natural products required for preclinical and clinical studies.

PARKE, DAVIS & COMPANY (N01-CM-43754)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under a bevy of environmental and stress situations; (2) an in vitro tissue culture assay laboratory which will assist in prescreening fermentation broths for cytotoxicity and will be used to help assay chemical fractions, fermentation improvement samples and large pilot plant lots more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

PENNSYLVANIA, UNIVERSITY OF (N01-CM-12188)

The objective of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems used are isolated heart and liver mitochondria, intact Ehrlich ascites cells and isolated, perfused organs. Using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores, an attempt is being made to identify effects which can be correlated with either the therapeutic activity or the limiting toxicities of antitumor drugs. This contract terminated in October 1974. See new contract number N01-CM-53830 for the current scope of work.

PENNSYLVANIA, UNIVERSITY OF (N01-CM-33719)

This contract provides, on a multiphase basis, for carrying out the design and installation of the Chemical Information System to handle a wide range of compound accessioning activities. This new system will provide improved capability to: (1) identify which compounds are new to the Drug Research and Development program, including the flagging of analogs and duplicates of compounds of interest; (2) produce computer-generated shipping documents and other communications to suppliers, screeners, in-house staff and contractors engaged in the acquisition, storage and distribution process; (3) maintain inventory control; (4) conduct on-line searches involving both structural and nonstructural information, using simplified inquiry procedures and providing structure output; and (5) maintain management and operational control of the system. The first two phases of this system, which provides the above capabilities have been installed. Two future phases are related to increasing the power of searching the files and outputting on-demand, formatted reports.

PENNSYLVANIA, UNIVERSITY OF (N01-CM-53830)

This contract is an outgrowth of a previous contract (N01-CM-12188). The object is to evaluate the acute and chronic effects of selected antitumor drugs on the energy performance status of isolated, perfused organs using both surface fluorescent and transmitted light absorbance methods developed by the Contractor. These models are to be applied to delineating the biochemical basis of either the therapeutic activity or limiting toxicity of selected drugs and their analogs.

PENNWALT CORPORATION (N01-CM-12309)

The objective of this contract is the synthesis of coordination compounds. Few transition metal coordination compounds have been adequately investigated as potential antitumor drugs. This contract will synthesize coordination compounds of platinum, rhodium, nickel, cobalt, copper and niobium. This area of synthetic investigation may be expanded to include additional classes of metal complexes if screening test results warrant.

PFIZER, INC. (N01-CM-53805)

The main purpose of this contract is to produce large quantities of primate type-C viruses in tissue culture and to purify and concentrate these viruses from the tissue culture fluids. In addition, the Contractor is to prepare certain viral components both to use in quality control of virus production and to provide the Project Officer with reagents to be used in the investigation of human neoplasms.

PHARM-ECO (N01-CM-23707)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 70% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range.

PHILIPS ROXANE LABORATORIES, INC. (N01-CM-12139)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxane Laboratories are packaged, labeled and shipped to the National Cancer Institute for subsequent distribution to clinical investigators.

POMONA COLLEGE (N01-CM-33703)

This project has two major objectives. First is the attempt to derive a structure-activity correlation for anticancer drugs. This will be done using

POMONA COLLEGE (NO1-CM-33703) (CONTINUED)

currently available data and the statistical methods of linear regression analysis. The second objective is the accumulation of fundamental data relating to hydrophobic bonding. These data will be used for further refinement of the structure-activity correlation as well as studies of the mechanisms of interaction of "mini-molecules" (drugs) with macromolecules (enzymes).

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PUBLIC SERVICES (TRANSFER OF FUNDS - YO1-CM-40003) (BALTIMORE, MARYLAND)

This Transfer of Funds permits service support of the National Cancer Institute, Intramural Baltimore Cancer Research Center program. This includes suitable laboratory facilities, support personnel and other administrative support.

RADIOBIOLOGICAL INSTITUTE (NO1-CM-53763)

The objective of this contract is to conduct preclinical investigations of the combination modality of radiation plus chemotherapy. Appropriate tumor test models are being explored and developed for this combination modality. With such models determination will be made of combinations and regimens of drug plus radiation that are optimal. The program is designed to provide an appropriate preclinical basis for the clinical application of radiation plus chemotherapy.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NO1-CM-33715)

This contract is designed to support the statistical and administrative efforts of the cooperative protocol studies of the Working Party for Lung Cancer.

RESEARCH TRIANGLE INSTITUTE (NO1-CM-92019)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the United States Department of Agriculture. The Contractor has many plants which are or have been in some stage of fractionation and several fermentation crude materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is used to expedite fractionation work.

RHODE ISLAND, UNIVERSITY OF (NO1-CM-43778)

This contract provides for the synthesis of nitrogen heterocyclic compounds with potential anticancer properties. The compounds being synthesized are analogs and derivatives of the pyrazoline and triazole ring system. The compounds have the potential to undergo a unique fission to produce metabolically active species that have a high probability of producing anticancer activity.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CM-22042)

The objective of this contract was to prepare and test analogs of the coenzyme form of Vitamin B_{12}. These consist of alterations of either base or sugar of the adenosine portion of the molecule or of alkyl cobalamins, by either chemical or enzymatic syntheses. After establishing identity of the agents, they were tested first for cell penetration and anti-B_{12} activity in vitro. Potentially useful agents were made in larger quantities for in vivo testing at the National Institutes of Health and/or Scripps. This contract terminated April 1975.

SHEEHAN INSTITUTE FOR RESEARCH (NO1-CM-23719)

The objective of this contract is the synthesis of new compounds with potential as Central Nervous System (CNS) antitumor agents. Specifically, compounds related to ergocornine, which are CNS active and which may pass the blood-brain barrier, are synthesized and evaluated in appropriate intraperitoneal and intracerebral test systems. Target-directed CNS drugs utilizing the carrier principle are also designed and synthesized.

SIDNEY FARBER CANCER CENTER (NO1-CM-43781) (FORMERLY CHILDREN'S CANCER RESEARCH FOUNDATION, INC.)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

SIDNEY FARBER CANCER CENTER (NO1-CM-43799) (FORMERLY CHILDREN'S CANCER RESEARCH FOUNDATION, INC.)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SIDNEY FARBER CANCER CENTER (NO1-CM-53839) (FORMERLY CHILDREN'S CANCER RESEARCH FOUNDATION, INC.)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer

SIDNEY FARBER CANCER CENTER (N01-CM-53839) (CONTINUED)

Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I study on Dianhydrogalactitol, NSC-132313 and high dose MTX thymidine rescue are proceeding.

SIMONSEN LABORATORIES, INC. (N01-CM-53817) (FORMERLY N01-CM-12101)

The Contractor maintains a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (N01-CM-53847) (FORMERLY N01-CM-23219)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (NIH-74-C-883) (FORMERLY NIH-73-C-816)

This contract furnishes approximately 312,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SIMONSEN LABORATORIES, INC. (NIH-74-C-884)

This contract furnishes approximately 52,000 six-week-old CD2F_1 (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CM-53820)

The object of this contract is to extend the techniques of in situ molecular hybridization to the presence of oncornavirus nucleic acid sequences in individual mammalian cells. To this end, tumor virus nucleic acids labeled to the highest possible specific activity are being tested for their usefulness for in situ hybridization.

SMALL BUSINESS ADMINISTRATION (N01-CM-43719)

The purposes of this contract are to provide supportive services in immunology, virology and tissue culture to the cancer chemotherapy program. The immediate goals are: (1) to develop a sensitive in situ immunological assay system for

SMALL BUSINESS ADMINISTRATION (N01-CM-43719) (CONTINUED)

detecting virus-related antigens in human leukemic cells as a potential diagnostic tool, (2) to extend and quantitate the effect of Rifamycin SV derivatives on reverse transcriptase and in preventing cell transformation *in vitro*, (3) to evaluate the *in vivo* metabolism of Rifamycin SV derivatives, and (4) to perform specialized cloning experiments of human leukemic cells and other human tumor cells of interest.

SOUTHERN ANIMAL FARMS (N01-CM-53845) (FORMERLY N01-CM-22013)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN ANIMAL FARMS (NIH-74-C-888) (FORMERLY NIH-73-C-863)

This contract furnishes approximately 336,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN ANIMAL FARMS (NIH-74-C-889)

This contract furnishes approximately 60,000 six-week-old CD2F_1 (BABL/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM-23241)

This contract was instituted to define the pharmacokinetics of adriamycin and guanazole in man. Initial efforts to define the metabolism and distribution of adriamycin in rabbits have been completed and submitted for publication. A theoretical model, based on the findings in the rabbit, is currently being applied to the clinical situation and should be completed in the coming fiscal year. This contract will be recompeted in FY-1976.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM-43795)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM-53842)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-02083)

The principal objective of this contract is to compare the relative toxicity and therapeutic activities of cancer chemotherapeutic agents alone and in combination when different dosage regimens are used in rodent tumor systems and in non-human primates or dogs. These studies are intended to serve as a basis for the design of a combination drug regimen acceptable for clinical trial. The combinations of adriamycin-cyclophosphamide and 5-fluorouracil-melphalan are currently under study.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-43756)

The overall objective of this multifaceted contract is to utilize experimental biological methods in vivo and in vitro to aid in the Division of Cancer Treatment in the discovery of more effective individual drugs and combinations of drugs, the optimal conditions for their use, and the study of combined treatment modalities in order to improve the control of human cancer. Tasks include primary and secondary screening in vivo and in vitro, detailed evaluation of active drugs, assessment of the potential value of combination chemotherapy and chemotherapy plus surgery, studies designed to provide information relative to mechanisms of drug action and the development of resistance, studies of factors influencing the growth and proliferation of tumor cells and the influence of chemotherapy or surgery on the growth kinetics of primary tumors as well as metastases, and the development of improved laboratory methods for the realization of objectives of the Drug Evaluation Branch.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-43762)

Southern Research Institute is one of the basic synthesis contractors for Drug Research and Development. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines, ring analogs of purines, nucleotides and nucleosides; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; streptozotocin analogs; aryl and heterocyclic triazenes; metabolites of cytoxan; and compounds containing very reactive chemical moieties.

SOUTHERN RESEARCH INSTITUTE (N01-CM-43780)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man. During the past year, studies have involved cyclocytidine, arabinosylcytosine, actinomycin-D, indicine-N-oxide and hexamethylmelamine.

SOUTHERN RESEARCH INSTITUTE (N01-CM-43784)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, projects are underway on (1) determining if new antitumor drugs have biochemical activities similar to those of clinically established drugs, (2) providing clear leads to biologically significant biochemical properties of new antitumor drugs, (3) evaluating the molecular mechanisms of action of new antitumor drugs, (4) developing systems to allow selection of structural analogs of clinically established antitumor drugs which have more desirable biochemical properties, and (5) developing biochemical rationales for the combined use of clinically established antitumor drugs.

SPARTAN RESEARCH ANIMALS, INC. (NIH-73-C-858)

This procurement contract provided for the supply of 104,000 $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for the Drug Research and Development compound evaluation program. Breeding animals originated in genetic centers. This contract terminated July 1974.

STANFORD RESEARCH INSTITUTE (N01-CM-12162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

STANFORD RESEARCH INSTITUTE (N01-CM-23200)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical

STANFORD RESEARCH INSTITUTE (NO1-CM-23200) (CONTINUED)

trials. The following investigations are conducted: (1) plasma levels and urinary, biliary and fecal excretion rates of drugs and/or metabolites of compounds of interest to the chemotherapy program; (2) tissue distribution of the parent drug and metabolites after oral and parenteral administration; (3) isolation and identification of the major drug metabolites; and (4) properties of the enzyme systems responsible for metabolism (subcellular localization, co-factor requirements and susceptibility to known inducers and inhibitors of drug metabolism). Both in vivo and in vitro studies are conducted using at least three animal species, including one species of sub-human primates.

STANFORD RESEARCH INSTITUTE (NO1-CM-33723)

This contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality and the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, liquid and gas), absorption spectroscopy (ultraviolet, visible and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases, methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NO1-CM-33742)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested.

Special attention is being directed to the study of the chemistry of the antibiotics, daunomycin and adriamycin, in order to develop agents of greater specificity at the molecular level. Analogs of daunomycin and adriamycin are being prepared and a total synthesis of adriamycin is under investigation. A modest effort is being extended toward preparing semisynthetic and simplified synthetic adriamycin analogs that retain only major features of the parent molecule. Also under investigation, is the synthesis of a group of compounds designed to intercalate into DNA in the manner proposed for several antitumor compounds and some unusual heterocyclics. Small efforts are directed towards preparation of analogs of ethidium bromide, 2,3-dihydroimidazo[1,2-b]pyrazole, formycin A and B, and the indole alkaloid ellipticine.

STARKS ASSOCIATES, INC. (NO1-CM-23203)

This service preparative contract supplies the Drug Development Branch with compounds required for drug evaluation studies, with 50% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multikilograms. The compounds prepared are not readily available in the quantities required on the open market or from the original supplier.

STARKS, C. P., INC. (NO1-CM-53745) (FORMERLY NO1-CM-23705)

This service contract supports the Drug Development Branch's efforts in the acquisition of chemicals and drugs for evaluation in the primary screen. The Contractor is required to: (1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; (2) collect samples; (3) provide support services in the processing of compounds; (4) aid in the management of computer-generated reports relating to compound acquisitions; and (5) assist in the development of methods for selecting materials for the screen.

TACONIC FARMS, INC. (NIH-74-C-864) (FORMERLY NIH-73-C-912)

This contract furnishes approximately 260,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TECHNICON INSTRUMENTS CORPORATION (NO1-CM-33746)

The purpose of this contract is to develop equipment for the rapid, alternated, individual determination of polyamines (spermidine, spermine and putrescine) in urine, whole blood, plasma or serum. This equipment will be designed to determine the sum of the polyamines in any of the above-mentioned biological fluids.

TENNESSEE, UNIVERSITY OF (NO1-CM-02084)

The objectives of this contract mainly include formulation development studies and small-batch production of oral, solid dosage forms. The Contractor also has the capabilities to prepare parenteral dosage forms in pilot-batch lots. All work is assigned by the Project Officer for the contract.

TEXAS INBRED MICE COMPANY (NO1-CM-53816) (FORMERLY NO1-CM-43711)

This genetic production center supplies a variety of inbred and hybrid rodents for tumor transplantation, for hybrid production (at Texas Inbred and other animal production contractors), and for compound evaluation studies. Animals are supplied from a colony of 14 strains of rodents.

TEXAS INBRED MICE COMPANY (NIH-74-C-927)

This contract furnishes approximately 153,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TEXAS, UNIVERSITY OF (N01-CM-53832)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of non-Hodgkin's lymphoma and metastatic soft-tissue sarcomas in adults. Studies are just beginning on this contract.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM-33710)

The objective of this contract is to identify clinical effectiveness of antitumor agents for the treatment of advanced ovarian carcinoma. This common tumor of women is known to be quite sensitive to a variety of alkylating agents as a class. The Contractor will randomize 100 previously untreated patients with Stage II or IV ovarian carcinoma to one of three choices of treatment. Melphalan, 5-FU and Hexamethylmelamine studies are nearly completed. All three drugs show significant activity.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM-61156)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents under development by the National Cancer Institute. During the current year, studies have been carried out on aspects of the pharmacology of the following agents: cyclocytidine, palmo-ara-C, benzoyl ara-C, asaley, ara-adenine, ara-6-mercaptopurine, 6-thioguanine, and the Yoshi dimethanesulfonate compound, bis (2-methylsulfonoxypropyl)amine (NSC-102627). Studies have been initiated on the effects of BCG and other immunoadjuvants on microsomal drug-metabolizing enzyme activity. A cellular pharmacology program has been initiated, utilizing human melanoma and lymphoma cell lines. The effects of antitumor agents are being evaluated in terms of parameters such as cell cycle stage sensitivity, cell kill as a function of duration of treatment, and repair of drug-induced injury. Studies under this contract are carried out in experimental animals and in cell culture lines, but are closely interrelated with Phase I clinical trials and clinical pharmacologic studies conducted at the M. D. Anderson Hospital and Tumor Institute and elsewhere.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM-12094)

This contract was designed to assess the value of protected environments in the chemotherapy of acute leukemia using appropriate controls for a quantitative

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-12094) (CONTINUED)

and qualitative comparison. In this study, a protected environment is defined as a laminar-air-flow room, used in conjunction with an extensive antimicrobial prophylactic regimen developed from previous studies.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-33737)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-43786)

The major emphasis of this project is to provide a detailed evaluation of the effects of new antitumor drugs on DNA biosynthesis or function, RNA synthesis, processing or function (ribosomal, messenger and transfer RNA) and incorporation into RNA or DNA.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-43801)

This contract was designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I clinical studies with Diglycoaldehyde, NSC-118994, Asaley, NSC-167780, Piperazinedione, NSC-135758, Ftorafur, NSC-148958, and Baker's antifol, NSC-139105, have been conducted.

TULANE UNIVERSITY (NO1-CM-53815) (FORMERLY NO1-CM-33701)

This primary genetic center serves to establish foundation colonies of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities. Foundation colonies are propagated in germ-free isolators, and small-scale production colonies are housed in bio-containment environments.

UPJOHN COMPANY (NO1-CM-43753)

This contract has for its primary objective the research and development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes, fermentation beers, including isolation, purification, characterization, production, biochemistry and pharmacology efforts.

The Upjohn Company has played a leading role in the development and exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation.

Major efforts in this contract are also devoted to biotransformation of known Upjohn antibiotics, studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program, and phases of toxicological and pharmacological studies within the capability of the Contractor and requested by the National Cancer Institute.

UTAH, UNIVERSITY OF (N01-CM-43806)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotics, tubercidin, sangivamycin and toyocamycin and other nucleoside antibiotics, (2) derivatives of the C-nucleoside formycin, (3) pyrazolo[4,3-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine nucleosides, (4) imidazo[4,5-*c*]pyridine, imidazo[4,5-*b*]pyridine and *v*-triazolo[4,5-*c*]pyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. This Contractor provides these difficult materials in quantities sufficient for *in vivo* antitumor evaluation by methods of synthesis developed by this group.

VALUE ENGINEERING COMPANY (N01-CM-33706)

Data processing services are provided by this contract to Drug Research and Development. The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, Drug Research and Development (including special queries of the master files via computer); (2) documenting all programs and contractor's procedures for data handling and running computer programs; (3) maintaining the computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) reducing and disseminating information on the receipt of compounds including those of particular interest to the Division of Cancer Treatment program; (5) expanding the present system to incorporate secondary testing not previously stored on the master files; (6) redesigning the existing system and developing new formats and methods of data collection; and (7) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data.

VALUE ENGINEERING COMPANY (NO1-CM-53821)

This contract provides for (1) programming support for the Drug Distribution and Cost Reporting System and the Clinical Drug Inventory System and (2) technical support for maintaining associated data files. These computer files serve as a record of all drugs sent from the National Cancer Institute to clinical investigators and include NSC numbers, drug name, lot number, as well as other data pertinent to the operation of the Drug Distribution and Cost Reporting System. In the past year, added emphasis was placed on upgrading and redesigning the system in keeping with the expansion of the Division of Cancer Treatment drug distribution activity.

VERMONT, UNIVERSITY OF (NO1-CM-33716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System to the Cancer Treatment area by integrating structured input ("cancer displays") into the system, thereby coupling learning with doing for all personnel who care for individual cancer patients entered on problem-specific plans (research protocols).

VETERANS ADMINISTRATION (YO1-CM-40004)

This program is a cooperative venture between the Veterans Administration and the National Cancer Institute for the clinical evaluation of potential anticancer agents.

VIRGINIA, UNIVERSITY OF (NO1-CM-12099)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

WALTER REED ARMY MEDICAL CENTER (YO1-CM-40005)

The primary objective of this interagency agreement is to provide support for Walter Reed Army Medical Center, Hematology-Oncology Service. This includes collaborative studies with Acute Leukemia Group B and the Medical Oncology area. Protocols are available for patients with acute lymphocytic leukemia, acute granulocytic leukemia, chronic lymphocytic and chronic granulocytic leukemia, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and brain, head and neck, breast and testicular tumors.

WARF INSTITUTE, INC. (NO1-CM-43707)

This represents a service contract to prepare extracts of plant materials for screening by the screening laboratories. A semi-quantitative test for alkaloids is run on plant materials of sufficient interest to be considered for fractionation. The majority of the Contractor's plants are obtained through a Transfer of Funds agreement between the National Cancer Institute and the United States Department of Agriculture. During the year, the special study of the extraction procedure was continued involving the preliminary fractionation, on a trial basis, of 500 plants in order to develop a procedure for increasing the yield of L1210-active plants. This study was successful and a new extraction procedure was adopted.

WARF INSTITUTE, INC. (N01-CM-53770) (FORMERLY N01-CM-60494)

This is a service contract for the *in vivo* screening of new, crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Currently, testing is being conducted at a level of approximately 50,000 tests per year according to Drug Research and Development protocols. The P388 lymphocytic leukemia is employed as the test system for crude natural products. Those that are active in this system are then tested in the L1210 leukemia. The L1210 leukemia is utilized for the testing of new synthetic materials. Other DR&D tumor systems may occasionally be used in the further testing of materials that were active in these systems or for analogs of materials that were active in these systems. Schedule dependency studies are conducted, upon request, for materials of potential clinical interest. Methodology and/or research and development, as well as special research programs, are scheduled as the need arises and are directed by the Project Officer.

WASHINGTON HOSPITAL CENTER (N01-CM-43710)

The objectives of this contract are to label Bleomycin with gamma-emitting isotopes for external counting, and to study the tumor localizing properties of this material for staging and diagnostic evaluation. Material has been shown in a preliminary manner, to localize in a higher proportion of tumors than other isotopes such as Gallium-67. The ability to identify tumor foci in the body will allow for more precise staging evaluation as well as for following changes in tumor size after treatment.

WASHINGTON STATE UNIVERSITY (N01-CM-22059)

This primary genetic center had as its objective the development of germ-free foundation colonies of inbred rodents. Pedigreed animals were derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring were artifically contaminated with pure cultures of non-pathogenic organisms and were developed as pedigreed expansion colonies in isolators. Offspring from this second stage were issued to secondary genetic centers, which in turn produced breeding animals for large-scale production colonies. The methods commonly accepted as best practice were followed with respect to environmental controls and microbiological monitoring. A small-scale production colony was maintained in order to provide limited numbers of rodents for special research and testing studies. This contract terminated November 1974.

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-43731)

The cytocidal effects of single drugs on normal hematopoietic stem cells and malignant cells (L1210, AKR, L cells) are quantitated by using a spleen colony or lung colony assay. Drug pairs are then evaluated for their effects on tumor and host cell survival. From these data principles for drug-pair sequence and dose intervals may be disclosed.

WISCONSIN, UNIVERSITY OF (NO1-CM-12275)

The principal objective of this contract is to study the mechanism of replication of genetic information, contained by RNA-tumor viruses, as well as the mechanism of integration of this information into the host chromosome. To accomplish this objective, studies are in progress with purified viral enzymes in order to understand clearly the enzymic reactions involved. The results on each isolated reaction will be assessed with relation to the physiology and life cycle of the intact virus. The DNA and RNA enzymes have been implicated in the etiology of human cancer. An understanding of the properties and mechanisms of these enzymes is, therefore, fundamental for the eventual solution of this problem.

WISCONSIN, UNIVERSITY OF (NO1-CM-33733)

This contract utilizing cell culture techniques provides a rapid assay as an aid to the purification of potential antitumor agents of plant origin. Fractionation products, prepared for the purpose of isolating the active *in vivo* constituent from crude plant materials and crude plant extracts, are tested for cytotoxicity. Procedures are those described by the Drug Research and Development protocols and methodology and special systems are carried out at the request of the Project Officer.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-12339)

The objective of this contract is to produce new anticancer agents based on their ability to intercalate and bind to nucleic acids. This would interfere with strand separation, which might be expected to result in suppressed or faulty replication of DNA or RNA. Evaluation of the agents synthesized is first by physical measurements of binding strength, melting temperatures, etc., and then by *in vitro* and *in vivo* studies.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-33711)

The objective of this contract is to provide in vitro screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of partially purified dihydrofolate reductase from L1210 leukemia cells, from human myelogenous leukemia and normal red blood cells. Special preclinical pharmacologic studies are carried out with selected agents, as is the gathering of appropriate data from patients receiving this type of drug.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-43797)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-53824)

The purpose of this contract is to fashion a chemotherapeutic attack, by means of new agents, on the neoplastic cell membrane, particularly on the synthesis of its glycoprotein components, aimed at either inhibition of membrane synthesis or alteration of functional aspects of the membrane such as antigenicity, cellular adhesiveness, and/or mobility, cell cycle determinants, or the state of cellular differentiation.

New agents, starting with modification of L-fucose, are synthesized after which their impact on a suitable number of the above-mentioned biological effects is determined.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-53843)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy vs. combination chemotherapy vs. single agent plus radiotherapy.

YESHIVA UNIVERSITY, ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CM-43787)

The major emphasis of this project is to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. For drugs having significant effects on protein synthesis, detailed studies on messenger binding, initiation, translocation and peptide bond formation will be done. In addition, detailed studies on the mechanism of action of new antitumor drugs which may directly affect the structure of DNA are performed.

Z01 CM 06401-04 ODCM
1. Office of the Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 Through June 30, 1975

Project Title: Data Processing Systems for Scientific and Management Information in the Division of Cancer Treatment

Previous Serial Number: NCI-6401

Principal Investigator: Meyer H. Gordon

Other Investigators: Anthony Kontaratos

Cooperating Units: DMB, DCRT

Man Years:

Total: 1.6
Professional: 1.2
Others: 0.4

Project Description:

Objectives

1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI and direct operations data input via OFM and the NCI Budget Office.

2. Revision of the DCT Stratification to provide comprehensive Disease-Oriented and Modality-Oriented budget information.

3. Establishment of a microfiche information system for archival and distribution purposes. Establishment of an on-line CRT display system for budget information.

4. Coordination of data pertaining to computer oriented expenditures within the Division of Cancer Treatment.

Methods Employed

1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B) and direct operations data is updated monthly at the computer terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (WYLGEN) for conversion to the appropriate transaction format. This update is designed for use by nonprogrammers. All entries in the file are classified with respect to the DCT Stratification and the basic priority

stratification of the DCT program (Linear Array, Supplementary Array, Exploratory Array).

2. New Stratification forms have been designed to capture additional Disease- and Modality-oriented budget information. Concurrently, programs have been written by DMB, DCRT to reinitiate the DCT data file by use of this new information. Contractual information procurement continues as before, with the TRW-generated linkage to the RCB, NCI contract management system for monthly updates.

3. The new NIH 5200 CRT terminal will soon replace the current IBM 2741 computer terminal in this office. This will allow for the development of on-line budget reports tailored to the 80 character CRT screen. Microfiche output will replace the current deluge of paper for better storage and distribution of information. Computer Output Microfilm (COM) has become a much-used option at DCRT.

4. With the cooperation of the Administrative Officers of DCT, data pertaining to DCRT accounts for DCT is now coordinated by this office.

Major Findings

1. Programs are being written by DMB, DCRT to generate reports by various combinations of the aforementioned classifications applied to contract and direct operations data as well as by DCT organization. In particular,several new reports will soon be available for classification of the DCT budget by the "new" DCT Stratification. Emphasis will be placed on Disease- and Modality-oriented summary reports. OFM will soon be providing DCT with reports that were previously done manually by the Administrative Officers. It is hoped that this data will be available for direct input to the DCT data file.

2. Regeneration of the DCT data file will allow for the incorporation of many new variables. Examples are: Subobject Class Code, Research Class, Profit/Non-profit, New/Renewal, Government/Non-government. Accordingly, many new budget reports may soon be generated upon request.

3. A CRT terminal will allow for better use of the Cancerline data base. An effort will be made to incorporate a "Keyword" potential in the DCT data file similar to that of Cancerline.

4. The availability of contract and DCRT account data has enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are now available via the "new" DCT Stratification as applied to the DCT budget.

Significance to the Program of the Institute

The computer system that is being developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system lead to a detailed description of the DCT research program.

Proposed Course

Possible modifications of the new stratification form to allow for the receipt of treatment data from other divisions of NCI is contemplated. These modifications will, in turn, require another large scale revision of the information system with the subsequent generation of new reports by DMB, DCRT. Programs will also be written to generate NCP approach category 6 reports via fixed relationshps with the stratification.

Automation of the retrieval of DCRT account data from DCRT tapes in the form required by the annual ADP plans will be investigated. This data combined with DCT stratification data will be used to generate reports of DCT data processing expenditures.

Keyword Descriptors: Data Processing, Management Information

Honors and Awards: None

Publications:

Gordon, M.H.: A Simulation Study of the Preclinical Development of Anticancer Drugs. Cancer Chemotherapy Reports. Part 3, Vol. 5, No. 1: pp. 65-78, Sept. 1974.

Z01 CM 06402-06 ODCM
1. Office of the Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 Through June 30, 1975

Project Title: Tumor Growth Kinetics and Chemotherapy

Previous Serial Number: NCI-6109

Principle Investigator: Stanley E. Shackney, M.D.

Other Investigators: Paul Bunn, M.D.

Cooperating Units: Dermatology Branch, DCBD

Man Years:

Total: 2
Professional: 2
Others: 0

Objectives:

1. To develop a model which will describe the growth of tumors in such a way that response to various chemotherapeutic agents can be simulated.

2. To apply the growth model to specific tumor systems.

3. To explore the pharmacodynamic interactions of chemotherapeutic agents in specific tumor systems with the aid of the model.

4. To develop an approach to chemotherapy of tumors based on the expected interactions between tumor growth kinetics and drug pharmacodynamics.

5. To collect cell kinetics data in man, and devise chemotherapeutic regimens based on cell kinetics principles which take these data into account.

Methods Employed:

Computer modeling techniques, *in vivo* and *in vitro* cell propogation techniques, radioautography with tritiated thymidine, flow microfluorometry and cell sorting.

Major Findings:

1. Modelling studies

In the previous year the computer model was applied to human kinetic data. Acute adult leukemia and malignant melanoma were studied in detail. Computer simulated populations were developed which yielded multithresholded PLM curves, interphase cell labelling indices and labelling intensities as a function of time, long term infusion mitotic and interphase cell labelling indices and mean grain counts as a function of time, all of which corresponded to the data actually observed in these two tumors. Significant kinetic differences were demonstrated between these two tumors:

i. Mean and median cell cycle times are longer in melanoma than in leukemia.

ii. The variance in the cycle time distributions was such that in melanoma there are fewer rapidly proliferating cells and many more slowing proliferating cells than in leukemia.

iii. The rate of DNA synthesis in the slowly proliferating melanoma cells is low and spread out over a long period. In leukemia, there is a relatively sharp peak of DNA synthesis toward the end of the cycle, even in cells with long cell cycle times.

iv. Melanoma contains a large fraction of nonviable cells which are cleared very slowly. The clearance of nonviable cells in leukemia is rapid.

Over the past year these solutions were tested to see if they were indeed optimal, and in particular to determine if the real data admitted interpretations other than those given above. Optimal solutions were found to be close to those obtained initially, and the differences between leukemia and melanoma demonstrated by the model can now be considered not only sufficient to account for the data, but necessary as well.

This phase of the modeling work is now complete. These studies will be presented in Copenhagen in May 1975, and a manuscript describing this work is in preparation.

The multiparameter simulation of sarcoma 180 in vitro was begun this year. This work is expected to receive major emphasis in the coming months, now that the simulation studies of leukemia and melanoma have been completed.

2. Experimental studies

a. PLM curve studies sarcoma 180 in vitro.

PLM curve studies in late log-phase-early plateau phase in sarcoma 180 in vitro which were completed last year, were submitted and published in Cancer Research this year.

b. Drug studies in sarcoma 180 in vitro.

In vitro - in vitro cloning studies were performed in log phase cells following Hydroxurea exposure over a five log range of concentrations and over a 12 hour range of drug exposure durations. It was found that lethality for this agent was a function of initial dosage, but not of drug exposure duration.

This is in contrast with cytosine arabinoside, where drug exposure duration appears to be a more important determinant of cell kill than drug concentration. The increased cell kill with increasing concentration of Hydroxyurea suggests that the duration of the "S" phase defined by Hydroxyurea lethality is no more discrete than the duration of S phase defined by ^{3}HTdR labelling.

Radioautographic studies and DNA content distribution studies were also performed in log phase, Hydroxyurea treated, S180 cells at two different drug concentrations and at two different drug exposure times. After a drug exposure of two hours, there was only a slight fall in the labelling index at low grain count thresholds, but the heavily labelled cell fraction was markedly reduced in comparison with controls. This was followed by a sharp rise in the heavily labelled cell fraction which was accompanied by a wave of cells traversing the "S" region of the DNA content distribution.

During 12 hour exposure to Hydroxyurea there was a fall in both the labelling index and the heavily labelled cell fraction in comparison with controls, and no changes were observed in the DNA content distribution. This is of considerable importance, in that it underscores the importance of radioautographic studies in interpreting DNA content distribution data. Another finding of major interest is that recovery in the rate of DNA synthesis was observed at 10 hours while the drug was still present. In separate studies we have demonstrated that medium from 12 hour exposed cells is capable of killing fresh cells, indicating that active drug is still present. This escape from Hydroxyurea effect has been observed by others, and may represent another important difference between this drug and cytosine arabinoside.

c. Experimental bone marrow studies.

In the previous year ^{3}HTdR studies in mouse bone marrow sections have suggested a considerable degree of kinetic-microarchitectural organization in this tissue, where heavily labelled, rapidly proliferating cells are found in the subendosteal region, and granulocyte differentiation and removal occurs in the central region. A paper describing these studies has been accepted for publication in Cell and Tissue Kinetics.

In the past year, these conclusions have been confirmed by stathmokinetic studies employing Colcemid, which demonstrated a preferential accumulation of mitoses in the subendosteal region.

During the course of these studies it was noted that marrow megakaryocytes increased with time following Colcemid administration. Dr. Paul Bunn has found that this increase is accompanied by a rise in peripheral platelet production. Increases in peripheral platelets in both animals and humans has been reported following the administration of Vincristine, another stathmokinetic agent. This drug was also investigated by Dr. Bunn, and a similar rise in peripheral platelets has been observed. The magnitude of the rise in circulating platelets is inversely related to dose for both Colcemid and Vincristine, where the greatest rises are seen at low doses. In the case of Colcemid, there is a marked reduction in marrow cellularity at higher doses; the effects of Vincristine on marrow cellularity are currently being studied.

These results have prompted us to consider the use of stathmokinetic agents at low dosage to protect the marrow in drug combination regimens. This approach appears to be particularly attractive in view of published reports of enhanced antitumor effects of cytosine arabinoside and Cytoxan given 12-48 hours after Vincristine in several experimental tumor systems and in man. Studies are currently under way to explore optimal relative doses and intervals for the Vincristine-Cytoxan combination in mice with respect to marrow toxicity.

3. Clinical studies

Serial skin biopsies and blood samples have been obtained in two patients with Sezary's syndrome who were given tritiated thymidine intravenously in a pulse dose. Multithresholded PLM curves and labelling indices as a function of time will be obtained from the latter and will be compared with the labelling index as a function of time of the cells in the blood. These studies will be repeated in the same patients following leukapheresis to see if recruitment can be demonstrated following a reduction of total body burden of tumor cells.

Initial attempts to translate kinetic principles into clinical practice are being carried out. A regimen has been devised for breast cancer consisting of the following 3 week cycle:

5FU-400 mg/M^2 (or more, as tolerated) i.v. day 1

Cytoxan 250 mg/M^2 (or more, as tolerated) P.O. QD days 1-3

Vincristine 0.075 mg i.v. days 11 and 15

Methotrexate 25 mg (or more, as tolerated) P.O. days 12 and 16

The rationale for this schedule incorporates the following principles:

i. Cytoxan and 5FU, which are the least proliferation dependent agents active in breast cancer are given early in the treatment cycle and methotrexate is given during the presumed recruitment phase.

ii. Methotrexate is preceded by low dose Vincristine in an attempt to spare platelet production.

This regimen has been given to one patient with breast cancer metastases to skin and bone who has failed two 10 cycle courses of FACP. After 4 cycles toxicity has been found to consist of nausea following the Cytoxan, and some mucositis during the methotrexate phase of the cycle. The WBC count has fallen below 4,000 on several occasions, but the platelet count has been maintained between 200,000 and 300,000 throughout. Skin metastases have been controlled, and are decreasing in size and number. Lytic areas of bone, which were not affected during FACP therapy, are showing increased density which the radiologists are interpretating as sclerosis.

Although data from one patient are hardly conclusive, they are encouraging in that they suggest that:

i. changes in drug schedule are well tolerated and may be effective, and

ii. pretreatment with low dose Vincristine may have a platelet sparing effect clinically.

The latter will be tested by leaving out Vincristine in alternate cycles.

4. Flow microfluorometry studies and cell sorting.

The Los Alamos flow microfluorometer and cell sorter was delivered in March 1975, and is operational at the present time. Computer software for the capture and analysis of raw four parameter/cell data will be delivered in July 1975.

Proposed Course:

1. Modelling studies

The multiparameter modelling of sarcoma 180 *in vitro* will be carried out over the coming year, now that detailed experimental kinetic studies in the unperturbed state have been completed. As detailed perturbation studies become available in this system during and after exposure to different drugs, the kinetic properties of these drugs will be modelled. Parallel studies on the kinetic behavior of sarcoma 180 *in vivo* are being carried out by Dr. Marc Straus at Boston University. It will soon be possible to model this system as well, and test the possibility that drug characteristics modelled from *in vitro* data are applicable under *in vivo* conditions provided that differences in cell kinetics and pharmacokinetics are taken into account.

2. Experimental studies

a. sarcoma 180 *in vitro*

Cloning studies, radioautographic studies, and DNA content distribution studies during and after cytosine arabinoside exposure are in progress, and will take about 6-9 months to complete.

With the arrival of the Los Alamos cell sorter, a new class of experiments has become possible, namely combined DNA content-radioautography studies on the same cells. These experiments should provide a well integrated view of the cycle age related effects of different drugs. Hydroxyurea and Cytosine arabinoside will be studied in this way, and the data will be compared with those obtained previously by other methods. Afterward, a broad spectrum of drugs will be studied.

b. Bone marrow studies

A study designed to localize spatially the cells in the marrow which are preferentially affected by Cytosine arabinoside *in vivo*, and to identify the region from which cells are recruited to replace those cells which are killed by ara-C is in progress. The experiment has been done and the marrows are being sectioned. The collection of grain count data will require the better part of a year to complete.

Studies designed to explore the effects of the Vincristine-Cytoxan combination on the marrow are currently in progress. Particular emphasis will be placed on finding the optimal interval and relative dosages which produce the least toxicity, and relating these findings to Vincristine's stathmokinetic properties and their effects on megakaryocyte production.

The Los Alamos cell sorter will be used to study fundamental aspects of granulocyte and megakaryocyte production. ^{3}HTdR labelling intensity as a function of time will be studied in relation to cell morphology and DNA content. For megakaryocytes, labelling will be studied in relation to ploidy. The effects of various drugs on the normal sequence will be studied.

3. Clinical studies.

Sezary's syndrome - In addition to multithresholded PLM curve studies and serial labelling index studies before and after leukophoresis, long term ^{3}HTdR infusions are also planned for at least two patients. When all the data are collected and processed, it will be possible to simulate this lymphoma on the computer, and compare results with those obtained in leukemia and melanoma. At that time it may also be possible to simulate chemotherapy on the computer and propose specific regimens based on these kinetic studies.

Hodgkin's and non-Hodgkin's Lymphomas - In discussions held with Dr. R. Young and C. Berard, interest was expressed in studying the lymphomas in relation both to their kinetic properties and their immunologic properties. Once basic techniques for processing human tumor specimens are standardized, the same samples will be split and studied by immunologic, conventional morphologic, and flow microfluorometric techniques. Selected patients will be given ^{3}HTdR and studied radioautographically as well. In those patients in whom serial biopsies are feasible the effects of chemotherapy on the cell size and DNA content distributions will be studied using flow microfluorometry.

The Leukemias - The marrow is easily dissociable into monodisperse cell suspension, and can be studied readily by flow microfluorometric techniques. Several reports have already appeared in the literature correlating DNA content distributions in leukemic patients with therapeutic response. Such studies could easily be performed at NCI, and could be combined with radioautographic studies in selected patients. Dr. Ziegler and Dr. Gralnik have individually expressed some interest; the feasibility of such studies will be explored further in the coming year.

Solid tumors - Malignant effusions can be studied readily using flow microfluorometric techniques. We have carried out some preliminary studies of ovarian ascites cells, and we are collecting samples for more detailed analysis. The monodispersion of solid tumors is of interest to us as well as to other groups across the country. We are also interested in performing both pulse and long-term infusion ^{3}HTdR studies in patients with multiple skin metastases, and we will be exploring the feasibility of such studies in the coming year.

Keyword Descriptors:

Cell Kinetics, Computer Models, Tritiated Thymidine, Autoradiography, Percent labelled mitosis curve, Hydroxyurea, Cytosine arabinoside, Colcemid, Vincristine, Cytoxan, Bone Marrow, Flow Microfluorometry, Cell Sorting, Sezary's Syndrome

Publications:

Shackney, S.E., and Ford, S.S. Correlations between the DNA Content Distribution and Tritiated Thymidine Studies in Relation to Population Size in Sarcoma 180 _in vitro_. Cancer Res. 34:1401-1407, 1974.

Shackney, S.E. The Radioautographic Transfer Function and its implications for Radioautographic Methodology. J. Nat. Cancer Inst. in Press.

Shackney, S.E., Ford, S.S. and Wittig, A.B. Kinetic Microarchitectural Correlations in the Bone Marrow of the Mouse. Cell Tissue Kinet. Accepted for Publication.

Z01 CM 07025-02 ODCM
1. Office of the Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 Through June 30, 1975

Project Title: Biomedical Engineering for Intramural Research, DCT

Previous Serial Number: NCI-7025

Principal Investigator: William Z. Penland, Jr.

Other Investigators: NCI: Seymour Perry, Paul Carbone, Michael Walker, Vincent Oliverio, Anthony Kontaratos, Kwang Woo, Stanley Shackney, Lewis Lipkin, Arthur Levine, Robert Graw, Joan Bull, Jeoffrey Herzig, Philip Waalkes, Philip Schein, David Poplack, Ronald Barr, Cephas Patch, Oleg Selawry, John Ziegler, Prem Sarin, John Wood, Frank Quinn, David Johns, Robert Gallo, Vincent Bono, Randall Johnston, Philip Pizzo, Ian Magrath, Richard Adamson
ADL: Alfred Wechsler, Leonardo Peusner, Phillip Thayer, Alan Burg, Edward Cook, Erica Drazen, Donald Lindsay, Robert Liss, Karl Wiig, Bruce Shapiro, Peter Kaiser, Lita Nelsen, Herbert Loeffler
RTI: Thomas Wooton, William Smith
NASA HDQ: Robert Zimmerman
ORNL: Julian Breillatt, Norman Anderson, Colin West, Carl Remenyik
U.S. Naval Weapons Center: Michael Benton, Douglas Sticht
Los Alamos Scientific Laboratories: Paul Mullaney, Harold Crissman, Gary Salzman
NIH Clinical Center: Gerald Johnston, Michael Green
LTD, NHLI: Robert Bowman, Ronald Atkinson, Peter Carmesi

Cooperating Units: Office of the Director, Division of Cancer Treatment Program Staff, DCT; Medical Oncology, DCT; Experimental Therapeutics, DCT; Drug Research and Development, DCT; Baltimore Cancer Research Center, DCT; Nuclear Medicine Department, Clinical Center, NIH: Laboratory of Technical Development, NHLI; Research Triangle Institute; Oak Ridge National Laboratories (ORNL); Arthur D. Little, Inc. (ADL); Sci-Med, Inc.; Abcor, Inc.; NASA, Goddard Space Flight Center; NASA, D.C. Headquarters; Litton Bionetics; Los Alamos Scientific Laboratories (LASL); Entropy Limited; Carnegie Mellow University; U.S. Naval Weapons Center; Stanford Research Institute

Man Years:

Total: 16.5
Professional: 15.5
Others: 1

General Objectives/Scope

1. To provide specialized and broadly based biomedical engineering (BME) resources for the Division of Cancer Treatment, NCI.

2. To identify and characterize problems amenable to BME solutions and to conduct BME research and development efforts responsive to the diverse research progams, projects and tasks of individual investigators of the Division of Cancer Treatment.

3. To develop and refine approaches for the effective management of broad BME resources through the contract mechanism.

Major Findings/Accomplishments

Sustained collaborative support in biomedical engineering, mainly of an exploratory/developmental nature, has addressed the feasibility of engineering concepts and methods; provided novel instrument research and development; and furnished analytical or model support and technical consultation for a variety of clinical and pre-clinical investigations of the DCT Program during this period. A widening range of engineering resources has been employed.

Instrumentation-Cellular DNA Studies

Major emphasis this period was given to the completion of a cell-analysis and sorting system applicable to tumor cell characterization and kinetic studies of DCT. This system, developed by the Los Alamos Scientific Laboratories, is capable of individual cell sorting at rapid rates (50,000 cells/minute) as determined by multiple cell parameters of size and fluorescence. The rapid accumulation of data of DNA synthesis patterns of the cell cycle both in the normal state and following drug administration will support NCI studies to (a) elucidate cycle stage dependence of drugs; (b) assist initial kinetic tumor classification; (c) speed up the acquisition of cell kinetic information needed in several intramural investigations; (d) permit clinical monitoring of various drugs in tumor and normal tissues; and (e) permit new studies of drug effects in _in vitro_ and _in vivo_ cell populations. The instrument and computer hardware was successfully developed and calibrated this period. It is now operational on a preliminary basis with NCI pending the availability of computer software for four parameter data acquisition and display. Biological assistance by LASL has stressed the experimental dispersal, fixation and staining of cellular material from patients and animal tumors of interest to future NCI studies.

ADL has explored both within and outside their company (Polaroid, University of Pennsylvania, SRI) techniques for fast measurements of DNA rates of synthesis. This relates to the development of new instrumental methods (above) for the kinetic characterization of cell populations of experimental and human tumors and their response to drug therapy. A collaborative project (NCI, SRI, ADL) will explore a technique that uses field emission mass spectrometry in conjunction with mutiple labelled isotopes. This project may lead to a method of measuring the average rate of DNA synthesis in 10,000 to 1000 cells. This degree of sensitivity when applied to the small number of cells sorted by the Los Alamos cell sorter will allow a two-dimensional analysis of cell cycle age and DNA synthesis rates on selected cell populations.

A collaborative project (ADL, DCT, DCBD) was begun to support subsystem trouble shooting, maintenance, and engineering improvements required by Los Alamos Cell Microfluorimenters located in two NCI laboratories (Shackney, Herman).

Instrumentation and SEM Studies - Leukocyte Procurement

Substantial progress has been demonstrated by the Oak Ridge National Laboratories (Breillatt) in collaboration with the NCI staff (Penland, Graw) with rotor development and with preclinical trials with dogs. As a result, the axial-flow rotor design with accessory control devices is approaching clinical trials during this period. Further development of back-up rotor systems of the radial-flow design (ORNL) and the centrifugal elutriator (ABCOR), has not been necessary. As a result of initial trials with dogs and of new insight into stresses exerted on the rouleaux by the plasma through which they sediment, interior flow path modifications were made and optimized by further testing and mathematical modeling; the volume flow rate of blood to the rotor has been automatically controlled in order to optimize rouleaux size and sedimentation rate; and an axial-flow design incorporating these changes for clinical testing during early FY76 has been initiated. The granulocyte recovery efficiency for the rotor in early dog trials was promising, averaging 70-75%.

Ideal operation of the axial-flow rotor requires control of two operating parameters, the hematocrit and interface position of the buffy coat. An approach to first measure and then automatically present a uniform hematocrit to the rotor separation chambers was conceived and reduced to practice (West, AEC, England). Initial trials of this control device using dogs have now led successfully to patient trials at the NCI and a series of improvements in sterility and electrical safety.

Investigation of a non-rotating seal (LTD, NHLI), which indicates advantages in platelet collection by centrifugation, was made (Penland, Breillatt) to examine the utility of this seal in current rotor designs of ORNL.

Transmission and scanning electron microscopy (SEM) methods were used this period (Liss, ADL) to define the processes of granulocyte adherence and elution from nylon fibers (Leukopak). While these new data have provided a clearer understanding of white blood cell/fiber interaction and of associated morphological changes in the processed cells, the isolation of possible factors affecting *in vivo* granulocyte circulation has been unrewarding. A series of experiments (Liss, Graw, Herzig), is now in progress to assess morphological damage and possible cellular repair that may be traced to the processing of fiber collected cells using various anticoagulants and elution techniques involving plasma or plasma-containing solutions.

Automatic Image Processing - Cell Identification

A feasibility study covering the integration of a computer controlled acousto-optic filter with differential interference optics (Cook, ADL), was completed this period to assist design decisions for an advanced microscope system for cell identification studies (Lipkin, DCBD).

A project (Kaiser, ADL) was completed this period to construct and test the assembler/compiler component of a cell modeling system (Lipkin) specifically designed for picture processing. The assembler/compiler represents a one-of-a-kind device to be connected and to control a unique array of process control devices, special scanner and microscope image data acquisition systems. In turn, the user will interact with the assembler/compiler via the DCRT PDP-10 system.

Initial development of a procedural description language (Shapiro, ADL) for use with the cell modeling system (Lipkin) was initiated this period. This project contributed part of the high level software development for the integration and control of optical and mechanical components by advanced picture processing techniques. Previous work during this period (Shapiro, ADL) established the utility of the Stanford Artifical Intelligence Language as a tool for the construction of cell description and analysis systems on the PDP-10, as well as for controls for a real time picture processor.

In support of the goal to follow the growth and division of cells in tissue culture by utilizing optical techniques and computer analysis (Lipkin) and to permit biochemical and pharmacological experiments on single cells, a design/development study (Peusner, ADL) of the flow system for the computer controlled tissue culture chamber will begin this period.

Engineering Support - Tumor Cell Biology

An ADL project (Lindsay) to conduct electron microprobe studies of cells to locate trace elements (Gallo, Levine) is in progress to exploit any enhanced sensitivity, speed, and spatial resolution of currently available electron microprobes, including the hybrid scanning-transmission microscopes (STEM), and the lastest generation of scanning electron microscopes equipped with analyzers for stimulated x-ray emission. Physical reference samples, similar to those originally devised by Bachmann and Salpeter to simulate trace-element-labelled substances in cells, have been prepared and tested as calibration standards for spatial resolution. These experiments, when complete, will represent an intermediate step between the detection of iodine in collected microscopic particles of AgI and the ultimate objective; the detection of localized concentration of iodine-labelled nucleic acids in cell sections.

A prototype device for automatic washing of filters in conjunction with the Millipore sampling manifold, model #3025, was designed and developed by ADL (Loeffler) in response to NCI laboratories (Gallo, Sarin) doing extensive hybridization studies. The unit is capable of automatic timing of washing periods for a preselected number of washes. A simple, reliable ball value limits the fluid fill of all reservoir chambers, and a vacuum pressure solenoid rotates the reservoir assembly to dump fluid simultaneously into the sampling manifold. Additional units are planned. The Millipore Corporation has demonstrated commercial interest in the device. To complement this effort, the value of commercial automatic pipetting devices is being investigated (Peusner, ADL).

Identification and quantification of the Epstein-Barr Virus in cells by electron microscopy (Liss, ADL) is in progress to support studies (MaGrath, Pizzo) to significantly increase the yield of this virus by various chemical and physical manipulation of the cells in which the virus is grown.

Mathematical Modeling - Cell Kinetics

The mathematical model (Woo) based on dividing the cell cycle into 4 cycling compartments and 2 non-cycling compartments for free growing tumors was improved to characterize specific experimental observations. In particular, changes in the cell cycle time, cell loss rate, and growth fraction as functions of the tumor size, were incorporated in the model. Subsequent simulation of a number of experimental tumors, compared favorably with experimental results. The tumor systems studied included Ehrlich ascites tumors, the JB-1 ascites tumor, L1210 leukemia, and Sarcoma 180, all in mice. The computational framework was also improved to a point where a full simulation can now be performed for about $.50. This is of value when repeated simulations are required to determine the relative merit of drug schedules.

Drug effects on experimental tumor growth were quantitatively analyzed in terms of cell kill (which is integrated with natural cell loss), cell cycle time elongation, and drug induced progression delay. These mechanisms were introduced in the mathematical model and characterized by computer simulation. Emphasis was given to the kinetic analysis of the effects of BCNU, ara-C, and MTX on L1210 leukemia.

The correspondence between the simulated results and the experimental data was good and conclusive for BCNU where the _in vitro_ data were well documented. This permitted a direct quantification of all parameters sufficient to verify the model by simulation of _in vivo_ experiments with no further adjustment of the model. More work is required to verify the ara-C and MTX models, although very good correspondence has already been obtained.

Results from these investigations and data obtained from the reference articles, together with mathematical studies and comparisons with several other available formulations, have been reported.

Further studies (Wiig, ADL) continue to support the application of mathematical and computational schemes to design possible treatment schedules for cancer therapy, and to provide insight into the specific use of the model to investigators in dealing with leukemia and solid tumors. This work will be based upon the multicompartmental model previously developed for the tumor-drug interactions. This effort will develop and synthesize theroretical drug dosage schedule which, through computer simulation, may demonstrate improved properties compared to initially devised schedules. Emphasis will be given to minimum dose and minimum average drug concentration as primary variables for measure of optimality. Trade-offs between variables of maximum cell kill and minimum drug concentrations, and their relationship to the effects on cell kinetics parameters, will be considered. Drug effects on the host, such as toxicity, etc., will not be treated initially.

Instrumentation - Cellular Kinetics

Feasibility trials were initiated with a commerical instrument (Artek, Inc.) to automatically scan and count autoradiographs and bone marrow cells with high precision in addition to counting bacterial colonies, its original function. Preliminary data comparing manual and machine counts of autoradiographs (Shackney, Ford) indicate excellent resolution of small grains and a variation between counts of <5%. Bone marrow cell counts of approximately 16,000 cells, required 10 minutes for machine counting in contrast to 20 hours for manual counting with no significant difference in counts. A review of grain counting requirements of laboratories sponsored by DCT to receive automatic grain counters (Lipkin) will be reviewed in view of the above findings. Also, a preliminary review of the signal-to-noise processing capability of this instrument is in progress (Penland, Herman, DCBD) to assess its potential as a low resolution pre-screening device for cervical smears based upon cell density and size informartion and other criteria for mass screening.

Instrumentation - Bone Marrow Colony Monitoring

A new project was initiated this period (Atkinson, Penland) to develop and evaluate prototype instruments for the quantitation of growth in colonies of marrow granulocyte precursors. The monitoring technique closely follows that developed in missle guidance systems (Sidewinder) for the remote detection of small targets against complex bright backgrounds.

New discrimination techniques and instrumentation are desirable to increase the clinical productivity of scoring colony forming units on a mass scale in support of studies to understand the hematopoietic regulation mechanisms and the assessment of abnormalities in white cell proliferation (Bull, et.al.). A survey (ADL) has revealed that no existing instrumentation is entirely adequate to monitor colony growth under a wide range of nutrient background conditions. Manual colony scoring as currently performed, involves a waiting period of ten days after plating before growth of the colony is registered.

This development project requires the Naval Weapons Center, China Lake, California to collaborate with laboratories of NCI and NHLI in the design and fabrication of three prototype instruments for initial evaluation in ongoing DCT studies. Relevant resources of NWC labs include extensive R&D backgrounds in similar but more sophisticated instrumentation and in the analysis and application of techniques for background discrimination problems relevant to tissue culture monitoring (reticle/optical system, signal processing, light polarization, etc.).

Collaborative NASA Developments

NASA information exchanges and hardware development in support of DCT investigators (Wooten, RTI), has involved three different projects in thermal control. A unique prototype freeze/thaw apparatus for bone marrow cryopreservation studies (Graw, Gorin) was re-designed this period at Goddard Space Flight Center to accomodate larger capacity blood cell containers and to improve thermal sensing and data recording. This improved device routinely provides very precise thermal cooling rates to 300 ml capacity containers by means of electrical heating and sensing elements built into conductive plates which are submerged in vapor phase nitrogen. In another effort, a special cooling helment for localized hypothermia will be furnished by the NASA Technology Utilization Office (Zimmerman) to NCI (Leventhal and others) for possible treatment of edema of brain tissue following radiation treatment. Finally, consultation regarding a disposable temperature indicator device and insulated shipping containers for a family of drugs (Davignon) was initiated this period.

Activity of Potential Clinical Interest

A new cooperative study (Liss, Bender, Johnston) was initiated by ADL and Dr. Carbone this period to develop new organ-specific compounds for γ-camera scanning of normal and neoplastic tissues, with particular emphasis upon studies of the pancreas. The experience of ADL in studying the distribution of more than 100 anti-tumor compounds in rodents using whole body radioautography represents the basis for screening organ specific compounds for use as γ-emitters.

ADL (Nelsen, Loeffler) has developed and demonstrated (DDR&D, MO, EXT) a perfusion device for the maintenance and perfusion of small animals in isolation. Commerical perfusion devices were found too large for the support of isolated organs of rats or comparable sized animals. Design emphasis was placed upon hollow fiber membranes for oxygenation to minimize priming volume and also upon simplified instrument construction and control. Initial tests will be conducted by Dr. Philip Schein.

To review advancing techniques in solid tumor imaging, several seminars and discussions have been held between the ADL staff, Nuclear Medicine Branch, C.C., Medical Oncology Branch, DCT, and commercial firms to review acoustical holography techniques, radiographic imaging with electron radiography techniques, multi-wire proportional counting chambers and other current developments from a variety of sources

Drug Activity Analysis

A pattern discovery program has been applied to extract physical/chemical features of drug compounds which show correlation with anti-tumor activity and toxicology. Results of this program are presently being evaluated for two groups of compounds; (1) samples of approximately 100-200 compounds of similar structure which have been tested for L1210 tumor activity, where the sample includes both active and inactive compounds, and (2) samples of 40-50 preclinically active compounds representing a wide range of dissimilar structures and known activities. The project is a joint effort by DRD Branch (Quinn, Wood, et.al.), Entropy Limited, the Carnegie Mellon University and ADL.

To determine the potential for drug detection and identification studies of in vivo materials (Adamson, Johns), field ionization mass spectrometry analysis of several chemotherapeutic compounds were conducted this period at Stanford Research Institute (Anbar). Analyses of esters of methotrexate were successful. Analysis of vincristine was not successful. Analysis of maytansine is incomplete.

A photoionization mass spectrometer, seen at the Institute of Molecular Biology, USSR,(Penland, Wechsler) has been review with current chemical ionization and conventional mass spectrometry techniques in the interests of furthering drug detection studies of DCT.

Major Consultations

DCT investigators were provided consultation on the following subjects principally by ADL specialists: Definition, Planning and Evaluation of Individual Biomedical Engineering Projects for DCT (Penland); Survey of Potential NCI Interest in Automated Cell Growth/Analysis System of Prof. D.A. Glaser, Berkeley (Perry, et.al); Experimental Evaluation of the Isomet Acousto-Optic Filter for Application to Automated Microspectrometry (Lipkin); Feasibility of Removing T-lymphocites from Patients with T-lymphoma Utilizing Affinity Chromatography Techniques (Graw); Feasibility of Using Ultrasonic Impediography to Nonivasively Monitor Tumor Growth and Regression (Penland, Johnston); Magnetic "Dust" Tracers as a Diagnostic Tool for Lung Diseases (Selawry, Plough, DCBD); Review of Electron Microprobe Techniques and Microchannel Plate Electron Image Amplifiers (Selawry); Feasibility of Chemical Thermal Indicators for Breast Cancer Detection (Selawry, Penland, DCBD); Application of Implantable Infusion Pump Utilizing Metal Bellows (Penland); Development of Flow-charts of DCT Instrumural Research Investigations (Kontaratos, Perry); Preparation of Affinity Chromatography Systems for Purification of Aldehyde Oxidase (Johns); Improvement and Automation of Cell Separation Technique (Barr, Perry); Collection and Concentration of Cells in Cerebrospinal Fluid Samples with Minimum Morphological Disturbance (Poplack); Project for Localization of SV40 T Antigen (Levine, Patch); Preparation of Templates for Early SV40 RNA by Electrophoresis (Patch); Experimental Techniques for Colloid Labelling of Macrophages (Poplack).

Significance

This biomedical engineering activity relates to a significant number of problems within the Division of Cancer Treatment that can potentially benefit from bioengineering approaches. This activity is successfully focusing diverse technical resources to support DCT laboratory and clinical research. It is broadening the scope and data base of the biological and medical scientist to achieve specific research goals. It thereby increases the probability that new engineering methodologies and instrumentation will contribute as in the past, to short and long range goals of the Division of Cancer Treatment.

Proposed Course

Major emphasis will be given to identifying key technical problems of DCT research programs that are amenable to BME approaches. To do his, physical scientists and engineers with multidisciplinary backgrounds and interests, who are new to the program, will be encouraged to investigate and contribute to current instrumentation and methodologies used in NCI laboratories and clinics. Special consultant teams outside of NCI may also be utilized. More attention will be given to particularly complex long term problems by technical assistance beyond present contract resources.

Specific biomedical engineering investigations and technical approaches are being generated in areas such as: drug development, evaluation and delivery; biochemical and molecular biological studies; blood cell therapy; patient protective environments; microwave heating of tumors; computer based models for support of cancer treatment; techniques for locating pulmonary tumors; improvements in the bioavailability of chemotherapeutic agents; cell culture models for solid tumors; and continuous flow devices for *in vitro* measurement of DNA rates of synthesis.

Keyword Descriptors:

Biomedical engineering, cell separation, cell kinetics, biochemical markers, analytical instrumentation, cell cytofluorimetry, cell sorting instrumentation, automation of biochemical assays, microanalytical techniques, EM/SEM characterization of cells, advanced tissue culture techniques, gamma camera imaging methods, blood cell procurement, blood cell preservation, centrifugal sedimentation rotors, computer controlled microscopy, bone marrow colony analysis, drug delivery systems, protective environment, biological isolation garments, pharmacokinetic modeling, computer techniques, automated microspectrometry, pancreatic, breast, lung detection, hyperthermia, microwave-tumor interaction, DNA synthesis rate measurement, rational drug development, DNA-RNA hybridization techniques, artifical intelligence techniques, grain counting, image processing,pattern discovery and recognition techniques.

Publications:

Technical reports are compiled on all appropriate biomedical engineering projects primarily for the use of the intramural staff and are furnished the National Technical Information Service, U.S. Department of Commerce. Publications, lectures, honors and awards, resulting from these efforts generally are reported under individual research projects and are obtainable from the Biomedical Engineering Unit, DCT.

Z01 CM 07085-03 ODCM
1. Office of the Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 Through June 30, 1975

Project Title: Quantitative Studies on Tumor Cell Kinetics and Biomarker Synthesis; and on the Dynamics of Cell Kinetic Parameters and Implications for Cancer Therapy

Previous Serial Number: NCI-7085

Principal Investigator: Kwang B. Woo

Other Investigators: Seymour Perry and M. Kim

Cooperating Units: School of Electrical Engineering,
Cornell University

Man Years:

Total: 1.5
Professional: 1.2
Other: 0.3

Project Description:

Objectives

1. To establish a quantitative relationship between tumor cells and their specific biomarkers. A mathematical model is developed which by using the concentration measured of a biomarker and the characteristics of its kinetic parameters, enables one to estimate the total number of tumor cells as well as any variation in number after therapy.

2. To determine the dynamics of cell cycle and proliferation kinetics and the effects of various antitumor drugs. The time course behavior of the cell age, cell size, and DNA content distributions of a tumor cell population is characterized by a discrete-time kinetic model employing computer techniques, and its implications for the design of therapeutic strategies are investigated.

3. To study by the formulation of metabolic reaction kinetics inhibitory activities of a number of antitumor drugs on specific enzymes in biosynthetic pathways, and to provide a better basis for optimum schedule of a drug or combinations of drugs for the treatment of leukemias and solid tumors.

Methods Employed

1. By treating each cell cycle phase as a separate compartment, a mathematical model has been developed and used to simulate the kinetic behavior of experimental tumor growth and associated changes in cell kinetic parameters. This

model has readily been extended to allow for biomarker synthesis by which a quantitative relationship is established between tumor cells and biomarkers in specific cell cycle phases. The model is capable of dealing with the effects of antitumor drugs on cell cycle kinetics and biomarker synthesis during tumor growth. The kinetic behavior of the model was investigated by computer simulation, and was favorably compared with experimental data of tumor cell populations, including: Ehrlich ascites tumors (Proc. nat. Acad. Sci. 56:1735-1742, 1966; Cell Tissue Kinet. 1:137-146, 1968; Cancer 29:261-266, 1972; Cancer Res. 29:1527-1537, 1969; Cell Tissue Kinet. 7:305-308, 1974), L1210 leukemia (Cancer Res. 27:2381-2385,1967; Cancer Res. 32:2452-2458, 1972, 34:3296-3302, 1974; Cancer Res. 31:402-408, 1971), JB-1 ascites tumor (Cell Tissue Kinet. 6:347-357, 1973, 7:47-60, 1974), and Sarcoma 180 (Cancer Res. 28:724-726, 1968; Cell Tissue Kinet. 6:165-172, 1973; Gann 61:563-568, 1970).

2. Utilizing the multicompartmental model, the effects of antitumor drugs on the proliferation of tumors have been investigated. The drug effects simultaneously considered are (a) non-cycle specific killing and cycle-stage specific killing of cells,(b) progression delay of cells through cell cycle phases, which may bring about accumulation of the cells in various phases of the cell cycle, and (c) prolonging cell cycle times; and these effects are analyzed in terms of possible variation in the behavior of cell kinetic parameters, namely cell cycle times, cell loss rate, and growth fraction and are implemented in the model specifying proper functional forms for the parameters. Simulation is carried out to examine the effects of BCNU, ara-C, and MTX on the cell cycle and proliferation kinetics of L1210 leukemia.

3. The distribution and synthesis patterns of polyamines in the spleen of mice during the growth period of L1210 leukemia have been studied by computer simulation of the multicompartmental model in which the activities of enzymes catalyzing the formation of polyanimes are specifically related to various stages of tumor growth.

4. A discrete-time kinetic model previously developed has been used to study the simultaneous occurrence of several processes in the growth of tumor cell populations by evaluating and analyzing the cell age, cell size and DNA content distributions of Ehrlich ascites tumor with the aid of the available experimental data. The experimental cell size distributions for the Ehrlich ascites tumor of various ages obtained by Andersson & Agrell (Virch. Archiv. Abt. B Cell Pathalogy, 11:1-10, 1972) were analyzed in detail.

5. A discrete-time kinetic model for chemotherapy has been developed to deal with the effects of antitumor drugs on the cell cycle and proliferation kinetics of tumor cell population in which the amount of antitumor drug administered is formulated to be the control function, and the transition matrix representing two modes of drug action, namely cell kill and progression delay or accumulation of cells due to drug, is characterized. A method of optimal drug dosage schedule utilizing an optimization scheme in engineering systems studies was introduced, which is designed to minimize the cost function measuring the effectiveness of drug dosage schedule and penelizing the use of toxic amounts of drug.

6. Analytical studies on control characteristics of allosteric enzymes involved in branched biosynthetic and amphibolic pathways have been continued and expanded to encompass drug inhibition in the biosynthesis of key metabolites in relation to tumor growth kinetics.

Major Finding

1. Tumor Cell Kinetics. The growth equation for the multicompartmental model was derived which is valid for all stages of tumor growth, provided that the parameters of cell cycle time, cell loss rate, and growth fraction vary appropriately with tumor size. The equation thus provides one with a framework within which he can test his hypotheses concerning the growth limiting mechanisms involved in tumor kinetics, and can be used as a consistency check to verify experimentally observed kinetic parameters.

The time-course behavior of three kinetic parameters, namely the cell cycle time, the growth fraction, and the rate of cell loss examined during the growth of tumor cell populations using the multicompartmental model was in good agreement with experimental data on Ehrlich ascites tumors, L1210 leukemia, JB-1 ascites tumor and Sarcoma 180. In order to gain further insight into the kinetics of Ehrlich ascites tumor growth, the model was utilized to carry out detailed analysis of the experimental data reported by Lala & Patt (Proc. nat. Acad. Sci. 56:1735-1742, 1966) and Schiffer & Markoe (Cell Tissue Kinet. 7:305-308, 1974) on cell kinetic parameters; and it revealed that the growth rate of Ehrlich ascites tumor cells as the tumor aged was significantly influenced by changes in the cell cycle time.

2. Tumor Cell-Biomarker Kinetics. The multicompartmental (cell cycle stage-specific) model was integrated with reaction kinetics of biomarker synthesis with reference to the L1210 tumor-polyamine system. Preliminary results indicated that the integrated model represents the overall relationship rather well, although further improvement is still needed in order to make it useful for relating with accuracy the concentration of polyamine experimentally (or clinically) measured to the number of tumor cells.

3. Drug Effects of Tumor Cell Kinetics. The relationship of drug-tumor interactions was established by extending the multicompartmental model, based on the dynamics of the kinetic parameters associated with the proliferating and the quiescent compartments. Three modes of drug action were considered: (a) cell kill, (b) progression delay or accumulation of cells, and (c) elongation of cell cycle times. First, for cell killing following drug administration, drug-induced cell loss rate was introduced which may be combined with the rate of natural cell loss. Second, progression delay was formulated by means of an accumulation of cells in the drug-induced quiescent compartments, which results in change in the growth fraction. Third, experimental observation that a progressive prolongation of generation time or S phase caused by increasing doses of antitumor drug is characterized through modification of the specific cell growth activities. The time-course of drug distribution in tumor-host system is also described by a two-compartment model and the factors affecting the affectiveness of drug action are quantitatively formulated as a function of

drug concentration. The effects of BCNU, ara-C, and MTX on the cell cycle and proliferation kinetics of L1210 leukemia, analyzed by computer simulation are favorably compared with available experimental data. Also investigated was the sensitivity of drug effects of variation in dosage schedule and different modes of drug actions exerted on each cell cycle phases.

4. Cell Kinetic Parameters-Cell Size and DNA Content Distributions. The growth dynamics of proliferating and non-proliferating cells described by discrete-time state equations were characterized by computer simulation in which the simulated results of the growth curve, the growth fraction, and the mean generation time were adjusted to fit the experimental data. The cell age distribution during the period of growth was obtained and by a linear transformation of the state transition matrices, was employed to specify the cell size and DNA content distributions. The time-course behavior of cell cycle parameters of Ehrlich ascites tumor was illustrated, and the parameters important for the transition of cells in the proliferating compartment to the non-proliferating compartment are discussed, particularly in relation to the G_1-G_0 and G_2-G_0 transitions as revealed by the variation of cell size distribution. It may be concluded from this study that the process of transition of proliferating cells to the resting states depends on the population size of the tumor. The latter in turn depends on the change in the growth medium and the increase in some type of growth inhibition brought about by the increasing size of the tumor.

5. A Discrete-Time Kinetic Model for Chemotherapy. The model has been extended from the one previously developed for cell cycle kinetics. Thus, cell kinetic responses of chemotherapy are represented in terms of perturbation of cell kinetic parameters - cell age, cell size, and DNA content distributions. The performance of the model, assessed by examining the effects of cell cycle stage-specific agents such as cytosine arabinoside on spontaneous AKR leukemia, compared favorably with experimental data.

An analytical method for determining the optimal single dosage schedule for cycle stage-specific drugs was presented. It introduces a cost function, called the performance index in systems theory, and then determines the dosage schedule which minimizes the cost function. The performance index is the weighed sum of the drug dosage at each application and the size of a tumor cell population resulting from the dosage, i.e., it measures the final size of a population as well as the amount of chemotherapeutic drug used to achieve the final size. The latter may be related to the undesirable effects associated with the toxicity to the host. The optimal drug dosage schedule designed for cytosine arabinoside in ARK leukemia was superior at the termination of therapy, although the non-optimal therapy schedule reduced the number of surviving tumor cells more effectively than the optimal schedule during the earlier therapy period.

6. Metabolic Control Mechanisms. The analysis of control activity of a number of key regulatory enzymes, which was previously carried out, illustrates the mechanisms between interactions of several metabolites. Efforts are under way to utilize such mechanism to deal with interactions of pairs of drugs on the growth kinetics of tumor cell populations.

Significance to Biomedical Research and the Program of the Institute

A quantitative relationship between tumor cells and a biomarker with reference to the kinetics of L1210 leukemic cells and polyamines, established by a mathematical mode, is of importance in evaluating therapy and designing therapy schedules, and can be extended to deal with biomarkers readily measurable in patients with various forms of cancer and leukemia. Such relationship may be developed for other types of tumor cells and corresponding biomarkers, which will require different forms of functional relationship for the kinetic parameters representing the control exerted by biomarkers on tumor cell proliferation.

The multicompartmental model for cell cycle kinetics and pharmacokinetics is very effective in examining into the kinetic behavior of key cell kinetic parameters responsible for retardation in tumor growth, and can provide insight into the time-course of perturbation of the parameters due to chemotherapy. One of the practical values of the model would be that it is readily expanded to integrate various metabolic reactions occurring in each cycle phase, including the known biochemical pathways, into the model and their perturbation resulted from administration of drugs. The schedule dependency of the model performance is of considerable interest, and further evaluation of the system should give some details of the dose-cell cycle kinetic relationship, which may be utilized in formulating a framework necessary for the design of optimal dosage schedule.

The availability of new experimental techniques to record rapidly the distribution of a large number of cells in the various phases of the cell cycle namely based on the cell size and DNA content distributions, may lead into the design of treatment schedules. A quantitative analysis of the cell age, cell size, and DNA content distributions can be carried out by the discrete-time kinetic model. In an application of the model, the growth dynamics of Ehrlich ascites tumor with the combined mode of the G_1-G_0 and G_2-G_0 transitions was predicted, and responses of chemotherapy in terms of perturbation of the distributions of DNA content as well as cell size were characterized. A method of optimal drug dosage schedule was developed by the application of an optimization scheme in engineering systems studies to the discrete-time kinetic model for chemotherapy; and the superiority of the schedule was evident at the termination of therapy, although the work is an initial step toward designing optimal schedules for single or combination drug therapy.

Proposed Course

1. Efforts will be continued to integrate the cell cycle and proliferation kinetics of tumor cells and the activities of enzymes responsible for biomarker syntheses and to characterize the effects of antitumor agents on the tumor-biomarker interactions. The model of L1210 leukemia-polyamine system will be modified to allow its further extension to other types of biomarkers for cancer. Different hypotheses and assumptions will be proposed and examined in relation to tumor growth mechanisms as a function of tumor cell population size and biological substances for cellular regulation.

2. The multicompartmental model of cell cycle stage-specific type and associated computer program developed of tumor-drug interactions (pharmacokinetic parameters combined with cell cycle parameters) will be improved to make it more effectively applicable for a wide range of tumor systems. In order to develop a framework for the design of optimal drug dosage schedules, attempts will be made to establish a quantitative relationship between cell kinetic parameters, namely cell cycle time, cell loss rate, and growth fraction, and drug effects expressed in terms of cell kill, progression delay, and/or elongation of cell cycle times. Emphasis will be given to deal with such notions as maximum cell kill, minimum dose and minimum average drug concentration as main variables for measure of optimality and their relationship to the effects on the cell kinetic parameters.

3. The pattern of DNA content distributions generated by the discrete-time kinetic model will be improved to account for distribution patterns observed experimentally which are typically given in combination of a Gaussian form due to the broadening effects introduced by electronic noise, light scattering, and also by nonuniform staining procedures. This will be used to evaluate the time-course of DNA content distribution and other related parameters, particularly DNA synthesis rate, during tumor growth as well as tumor regression by therapy. Efforts will also be continued to investigate the cell kinetics of animal and human solid tumors and the effects of antitumor agents in cell kinetic behavior which are to be represented in terms of parameters of the model and to utilize the result for designing optimum scheduling of antitumor drugs.

4. A kinetic equation will be derived to analyze inhibitory activities of antitumor drugs on DNA polymerases. Patterns of the interactions of drugs that inhibit steps of biosynthetic pathways of key metabolites will be investigated; and kinetic studies of the interactions of two or more antitumor drugs will be carried out in relation to the growth of tumor cells and the synthesis of metabolites.

Keyword Descriptions:

Mathematical modeling, computer techniques, biomarker synthesis, cell cycle kinetics, pharmacodynamics, cancer therapy

Honors and Awards:

Kwang B. Woo

1. "Time-domain Analysis of Conformational Changes in Proteins." Presented at the 18th Annual Meeting of Biophysical Society, Minneapolis, Minnesota, June 1974.

2. "A National Targeted Research Program Plan and Implications for University Research." Presented at Integrated Symposium on Science and Engineering, Seoul, August 1974.

3. "Theoretical Analysis and Computer Simulation of Ehrlich Ascites Tumor." Presented at the 27th Annual Conference on Engineering in Medical and Biology, Philadelphia, Pennsylvania, October 1974.

4. "Analysis of the Effects of Antitumor Drugs on Cell Cycle Kinetics." Presented at the Annual Meeting of APhA Academy of Pharmaceutical Sciences, Symposium on "Pharmacokinetic Modeling of Anticancer Drugs," New Orleans, Louisiana, November 1974.

5. "A Quantitative Study of Tumor Cell Kinetics and Chemotherapy by the Discrete-time Kinetic Model." Presented at the 19th Annual Meeting of Biophysical Society, Philadelphia, Pennsylvania, February 1975.

Publications:

1. Kim, M., Bahrami, K., and Woo, K.B.: A discrete-time model for cell age, size, and DNA distributions of proliferating cells, and its application to the movement of labeled cohort. IEEE Trans. Biomedical Engineering 21:387-398, 1974.

2. Woo, K.B., and Wiig, K.M.: Effect of antitumor drugs on the kinetics of tumor cell-biomarker relationship. In Hampton, J.C. (Ed.): The Cell Cycle in Malignancy and Immunity. ERDA Symp. Series (CONF-731005), Springfield, Virginia, NTIS, U.S. Department of Commerce, 1975, PP. 485-503.

3. Kim, M., Bahrami, K., and Woo, K.B.: Mathematical description and analysis of cell cycle kinetics and the application to Ehrlich ascites tumor. J. theoret. Biol. in press.

4. Kim., M, and Woo, K.B.: Kinetic analysis of cell size and DNA content distributions during tumor cell proliferation: Ehrlich tumor study. Cell Tissue Kinet. 8:199-220, 1975.

5. Woo, K.B., Brenkus, L.M., and Wiig, K.M.: Analysis of the effects of antitumor drugs on cell cycle kinetics. Cancer Chemother. Rep. in press.

6. Woo, K.B., Wiig, K.M., and Brenkus, L.M.: Variation of cell kinetic parameters in relation to the growth rate of Ehrlich ascites tumor. Cell Tissue Kinet. in press.

This report was previously entitled "Kinetic (Quantitative) Studies on Tumor Cell and Biomarker Interactions; and on Cell Age, Cell Size, and DNA Content Distributions and Their Implications for Cancer Chemotherapy".

Z01 CM 09000 ODCM
1. Office of the Director
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 Through June 30, 1975

Project Title: Hemopoietic Stem Cells in Human Peripheral Blood

Previous Serial Number: None

Principal Investigator: Ronald D. Barr, M.R.C.P., M.R.C. Path.

Other Investigators: Jacqueline Whang-Peng, M.D., Gregory Sarna, M.D., Seymour Perry, M.D.

Cooperating Units:
1. Massachusetts Institute of Technology
2. Tumor Cell Biology, DCT, NCI

Man Years:

Total: 2.1
Professional: 1.1
Others: 1.0

Project Description:

The objective is to identify and characterize that discrete population of nucleated blood cells, which exhibits true pluripotentiality and the capacity for unlimited self-renewal. Peripheral white blood cells are separated into morphologically homogeneous fractions, by a combination of physical and immunological techniques. Predominant among these are the methods of isopycnic sedimentation and velocity sedimentation at unit gravity, non-specific differential leucocyte adherence, and the determination of functionally separable lymphocyte subpopulations, based on differences in surface membrane characteristics, detectable by rosette formation with a variety of preparations of xenogeneic erythocytes.

Cell suspensions thereby obtained are cultured in vivo, in millipore diffusion chambers, implanted intraperitoneally in lethally irradiated mice. Attempts to metabolically and functionally characterize the purified cell fractions include an evaluation of the lysozomal acid hydrolases: betaglucuronidase and leucocyte acid phosphatase, and estimation of the DNA metabolising enzymes: terminal deoxynucleotidyltransferase and 'true' DNA polymerases. To accomplish the latter objective preliminary studies are performed on the thymuses of leukemic AKR mice to assess the possible influence of proliferative cell cycle stage on enzyme activity.

Hemopoietic stem cell activity is found to reside in lymphocytes which are significantly larger than the majority and which fail to show the rosetting reactions of thymus-dependent (T) and bursa-equivalent (B) cells. On in vivo culture these cells proliferate, as evidenced by an increase in total cell number, and differentiate to all three main hemopoietic cell lines - benzidene-positive normoblasts; platelet-producing megakaryocytes; and granulocytes and monocytes, which are confirmed cytochemically by appropriate esterase reactions and metachromasia with toluidene blue (basophils). These cells probably account for only 1 in 10^5 - 10^6 total peripheral blood leucocytes and their cytological appearance on light microscopy may be characteristic. To date there is no evidence that they are metabolically distinguishable from other components of this tissue. The activity of the lysozomal acid hydrolases appears to be related simply to cell size. Terminal transferase is detectable at high levels in leukemic AKR murine thymus, but does not vary with cell cycle stage. Essentially there is no demonstrable enzyme activity in pure T cells, pure T cells cultured with phytohemagglutinin, pure B cells and stem cell concentrates. Nuclear DNA polymerase is likewise unrelated to cell cycle stage but the cytoplasmic enzyme activity is strikingly related to the phase (S) of DNA synthesis, showing a greater than ten-fold increment when estimated in the murine tissue. Furthermore, this moiety is all but absent from G_0 cells.

As a result of these findings it is hoped that the understanding and control of the effects on hempoiesis of the several modalities currently employed in cancer therapy, will be enhanced, and the ease and efficiency of stem cell transplantation improved. In a broader biomedical context, meaningful studies, in the human organism, of the basic processes of proliferation and differentiation, are considerably facilitated.

Pursuit of these studies will involve identification of radioisotopically labelled progeny of stem cells by chromium autoradiography; determination of radiosensitivity and the effects of cell cycle phase specific drugs; evaluation of the influence of other cell fractions on stem cell growth; and attempts at perturbation of the growth kinetics of this material by erythropoietin and pharmacological agents.

Keyword Descriptors:

Hemopoietic stem cells; millipore diffusion chambers; DNA metabolizing enzymes; cell kinetics

Honors and Awards:

1. Barr, R.D., Whang-Peng, J., Perry,S.: Recognition of the Human Hemopoietic Stem Cell. Presented at the Annual Meeting of the American Association for Cancer Research, San Diego, California, May 1975.

2. Barr, R.D., Whang-Peng, J., Perry,S.: Recognition of the Human Hemopoietic Stem Cell. Presented at the Annual Meeting of the Professional Association of the U.S. Public Health Service, Las Vegas, Nevada, June 1975. J.D. Lane Award Finalist.

3. Perry, S. - Presented First Jenaro Haddock Memorial Lecture, Studies of Leukocyte kinetics and Relevance to Chemotherapy, at invitation of Puerto Rican Society of Hematology, San Juan, April 8, 1975.

4. Perry, S., Sarna, G., and Omine, M.: Cytokinetics in Acute Leukemia Before and After Chemotherapy. XV Congress of the International Society of Hematology. Jerusalem, Israel. (Sept. 1-6) 1974.

Publications:

1. Sarna, G.: The Resting Cell: A Chemotherapeutic Problem, Part 1 and Part 2. Biomedicine 20: 322-326, 384-389, 1974.

2. Perry, S.: Human Leukemia - An Overview in Modern Trends in Human Leukemia, R. Neth, R.C. Gallo, S. Spiegelman, F. Stohlman, Jr. (Eds) J.F. Lehmanns Verlag, Munchen, pp. 6-19, 1974.

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT (DR&D)

DIVISION OF CANCER TREATMENT (DCT)

Drug Research and Development has as its primary task the search for and development of new and potentially more effective anti-tumor agents, as well as the development of better methods of drug selection and prediction of optimal therapy with new and old drugs and drugs combined with other treatment modalities. With respect to the Division of Cancer Treatment (DCT) Linear Array, the organization is responsible for all preclinical work in the development of drugs except that concerned with toxicology and pharmacology.

The DR&D program includes both in-house laboratory operations and contracts with pharmaceutical and chemical companies, research institutes and academic institutions, with the major portion of the funding directed toward the contract-supported activities. The program depends heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts. The program activities cover the following specific areas:

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
3. Development of new agents from fermentation, plant and other natural products.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Primary screening and evaluation of new materials.
7. Bioassays, both *in vivo* and *in vitro*, to monitor the purification of fermentation and other natural products.
8. Study of optimal drug routes and regimens, including therapy with combinations of drugs and combined modalities.
9. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential anti-tumor agents.
10. Investigation of mechanisms of action and resistance of drugs of clinical interest.
11. Animal procurement, distribution and disease control.
12. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
13. Development and operation of data processing systems for both biological and chemical information.
14. Publication of the journal, *Cancer Chemotherapy Reports*.

The input of synthetic compounds to the primary screen continued to increase in 1974 as a consequence of decisions made by the Division of Cancer Treatment in prior years (Table I). Because of the use of the mini-screen, the screening of these materials was carried out without a significant increase in the resources devoted to screening. The mini-screen will be discussed in greater detail later. The decreased input of plant products during 1974 in comparison to 1973 reflects the deliberate effort to balance this aspect of the program because of the relatively high cost of developing such materials. In view of the improved yield of active crude extracts resulting from the new extraction procedure described last year, the lower input will not result in a lower overall yield of active materials. The lower apparent input of fermentation products is a consequence of the greater emphasis on *in vitro* prescreening, thus resulting in fewer materials being submitted for the *in vivo* screen.

Returning to synthetic compounds, the level of input is expected to drop substantially during the next year for a number of reasons:

1. Such a high level is exceedingly difficult to maintain even if it were desirable.

2. More effort will be devoted to the "pre-selection" of materials possessing the highest probability for demonstrating activity, to the extent that the state of the art permits such pre-selection.

3. A significant effort is expected to be devoted to methodological studies relating to the evaluation of new experimental models, and this will of necessity be done at the expense of primary screening of synthetic compounds. It is not known at this time what level of input will be maintained in 1975.

The number of tests carried out in 1974 also showed an increase (Table 2). As was indicated in last year's report, there has been an apparent decrease in the yield of active compounds utilizing the mini-screen procedure. An exhaustive analysis was carried out during 1974 in an attempt to determine if in fact the yield was lower and if so what the reasons for this may be. Since the mini-screen procedure was utilized only for new structures while analogs were tested by different protocols, it is somewhat difficult to determine the actual effect of the mini-screen on the overall yield, since in earlier years the analogs and other materials were tested by the same protocol and overall yields did not differentiate between the two types of materials. It does appear, however, that the yield did indeed decrease, and that this occurred coincidentally with the advent of the mini-screen procedure. Although it is not possible to determine at this time the precise reasons for the decreased yield it appears likely that one of the major factors is related to the genetic makeup of the animals used for screening. Minor histocompatibility differences among inbred animals from different sources have been observed. This fact, and the inherently greater variability of the mini-screen utilizing smaller numbers of animals, may have combined to decrease the degree of reproducibility and create some of the problems that have been apparent. As a result of these observations, two changes in the animal program have been made. First, the animals used for screening will be shifted almost entirely to the CDF_1 hybrid which is more compatible with the L1210 and P388 leukemias than is the BDF_1. Second, arrangements have been made to provide a continuing supply of primary genetic stock to the genetic centers from NIH and no other source. This will eventually result in a "purification" of the genetic integrity of the mouse supply, but will not be completely effected for about 1 1/2 years. In the meantime, it appears that the mini-screen can be used effectively by decreasing somewhat the cut-off point for active materials at the first stage and then utilizing a larger number of animals for confirmatory testing. Modified procedures utilizing these changes in protocol have now been put into effect and it should be possible within the next few months to determine whether these changes have improved the overall yield of active compounds.

As a result of the screening and related drug development efforts, seven materials passed Decision Network 2A in 1974. That is, they demonstrated sufficient biological activity and chemical or other uniqueness to warrant the effort in dose formulation development, etc., leading to preclinical toxicology and clinical trial. These materials are listed in Table 3. The coordinated preclinical drug development work in 1974 resulted in three IND applications being filed, signifying the initiation of clinical trials. These materials are listed in Table 4.

OTHER SELECTED HIGHLIGHTS

1. In collaboration with the World Health Organization a world conference on screening was held in Geneva in September 1974. Participants from a large number of countries presented data on the experimental screening systems utilized in their respective laboratories. As a result of the discussions held at that meeting, it was agreed that the L1210 leukemia would be used as a standard reference system in all laboratories. Thus, even though each laboratory will maintain its own spectrum of tumor systems for screening and/or other purposes, the investigators agreed to evaluate their materials of interest in the L1210 leukemia in order that a single frame of reference could be applied to any new drug discovered throughout the world. In addition, the investigators agreed to test a group of standard agents in their own systems in order to gain a better understanding of the relative sensitivities of this wide variety of experimental tumor systems. NCI agreed to supply the L1210 leukemia and the standard reference drugs to all of the institutions willing to participate in this cooperative effort. It is believed that by this procedure it will be easier to evaluate the relative importance of new drugs that may be discovered as active in systems not used within the NCI program, to facilitate the duplication of results found by other investigators throughout the world, and to identify other experimental systems that might be of interest as potential screens.

2. The heightened interest in Methotrexate, because of its demonstrated utility in high dose regimens for the treatment of osteogenic sarcomas and other cancers following surgery, has placed a demand for both larger quantities and higher quality of the drug. The Drug Development Branch, through its contract at Southern Research Institute, has achieved a breakthrough by developing a new, improved synthetic procedure. The new method is rapid, relatively simple, and provides the drug in high purity at a lower cost.

The method is being utilized successfully for large-scale production by other NCI contracts. A patent application has been filed.

3. Studies on the chemical modification and new analog development of the alkaloid vincristine have been lacking due to the complex chemical nature of this compound. A major breakthrough has been made by our contractor at the University of British Columbia. A synthetic procedure which provides the correct stereo-chemistry at the bridging carbons of the dimeric halves of the vinca alkaloids has been discovered. This will provide both vincristine and analogs with the natural chemical configuration. A patent application has been filed.

4. During 1974, the Mammalian Genetics and Animal Production Section continued to meet the ever-changing requirements of the screening program. In this regard, about 1 million inbred mice and 3 1/2 million hybrid mice, in addition to smaller numbers of other animals, were provided.

5. Cancer Chemotherapy Reports continued to establish its position as a major primary journal in the cancer field. In 1974, 6 bimonthly issues of Part 1 were published, involving a total of 978 pages. In addition, 4 issues of Part 2 and 1 of Part 3 were published. The number of paid subscriptions continued to rise.

6. Because of the steadily increasing effort involving the clinical evaluation of new and established drugs, the development, production and procurement of clinical dosage forms has continued to increase. During 1974, this involved 7,554,533 formulated units, including 5,038,270 compressed tablets, 522,750 hard gelatin capsules, and 1,993,513 vials.

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During the past year, Drug Research and Development noted with sincere regret the retirements of Mr. Samuel Poiley and Dr. Jonathan Hartwell, both of whom completed long and distinguished careers at NIH and NCI. They will be greatly missed by their associates and collaborators throughout the world, although fortunately both are continuing to maintain informal contact with the staff.

Table 1

Input and Output of

Drug Development Program

	Calendar Year								
Materials Submitted	1966	1967	1968	1969	1970	1971	1972	1973	1974
Synthetic & crystalline natural products	8,008	7,413	10,915	9,820	8,521	10,151	15,770	34,143	39,540
Crude fermentation products	4,284	4,982	4,256	2,184	2,172	1,215	2,712	2,550	725
Crude plant products	3,620	6,253	4,245	5,109	3,499	3,015	10,434	11,661	5,830
Animal products		446	641	757	587	1,203	1,669	2,689	2,011
Total	15,912	19,094	20,057	17,870	14,779	15,584	30,585	51,334	48,380
IND applications filed	3	4	5	5	6	8	5	13	3

Table 2

Screening Tests

	Calendar Year						
	1968	1969	1970	1971	1972	1973	1974
L1210 Lymphoid Leukemia	107,445	80,545	79,892	102,424	103,681	133,481	211,832**
P388 Lymphocytic Leukemia	14,903	43,223	42,270	53,449	180,027	198,711	124,396**
Walker Carcinosarcoma 256	44,563	20,328	10,247	4,368	2,770	--	909
B16 Melanoma	286	1,240	1,342	1,423	6,028	15,989	11,724
Lewis Lung Carcinoma	244	925	3,033	2,803	4,141	2,441	2,770
AK Leukemia							925
Other Tumors	774	426	504	1,780	6,458	5,493	2,821
Total *In vivo*	168,215	146,687	137,288	166,247	303,105	356,115	355,377

** Includes tests against Resistant Tumor Lines; 970 in L1210 and 432 in P388

Table 3

Compounds Passing Decision Network 2A

NSC No.	Compound Name	Source
143,095	Pyrazole-3-carboxamide, 4-hydroxy-5-β-D-ribofuranosyl- (Pyrazomycin) (Pyrazofurin)	Fermentation
157,365	Neocarzinostatin	Fermentation
163,501	5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro- (AT-125)	Fermentation
170,105	Macromomycin	Fermentation
172,755	Glycine, N-[1-oxo-5-(1H-Purin-6-ylthio) pentyl]-, ethyl ester (Butocin)	Synthetic
176,319	Quinolinium, 6-amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]phenyl]amino] carbon-yl]phenyl]amino]-, dibromide	Synthetic
178,248	D-Glucopyranose, 2[[[(2-chloroethyl)-nitrosoamino]carbonyl]amino]-2-deoxy- (chlorozotocin)	Synthetic

Table 4

IND'S Filed

NSC No.	Compound Name	Source
132,313	Galactitol, 1,2,5,6-dianhydro-	Synthetic
220,537	Corynebacterium Parvum Vaccine	Fermentation
B116,327	Mycobacterium Tuberculosis Vaccine (BCG) (Connaught)	Fermentation

Project No. Z01 CM 07101-01
1. Office of Associate Director
for Drug Research and Development,
Division of Cancer Treatment, NCI
2. --
3. Silver Spring, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computer Methods for Drug Pre-Selection Based on Structure-Activity

Previous Serial Number: None

Principal Investigator: Louis Hodes

Other Investigators: G. Hazard and S. Richman, Drug Development Branch, DR&D, DCT, NCI; A. Guarino, Laboratory of Toxicology, EXT, DCT, NCI; A. Feldman, Walter Reed Army Medical Center; K. Chu, Division of Computer Research and Technology, NIH

Man Years:

Total: .5
Professional: .5

Project Description:

The chemical structure and biological test data that have been systematically collected over the years should be the basis of an automated method for selecting from potential drug accessions those compounds with high probability of antitumor activity, and also selecting the tumor system likely to demonstrate this activity. Such a system will help optimize the utilization of screening resources. The decision-making power of the computer will be used to correlate molecular structure and other physical/chemical properties with known activity in various test systems. At first such a system will aid in pre-selecting drugs for screening. Later such methods may play a more active role in seeking additional compounds which are promising or not yet adequately represented in our files.

Specific Objectives:

1. Check out the feasibility of performing structure-activity correlations on a large scale.

2. Develop a method that has a good chance of succeeding on a volume of 25,000 new drugs per year with known data on 250,000. The method must have sufficient power so that the expected loss in good drugs will be compensated by the gain in power for screening computer pre-selected compounds.

3. Develop a plan for carrying out such a method. This will undoubtedly be in stages.

4. Do Step 3 above.

Methods Employed:

Several methods from pattern recognition and statistics have been considered. These include learning machines, nearest neighbor matching, cluster analysis and Bayesian conditional probability methods. As structure data, molecular fragments are being used.

Major Findings:

1. Feasibility

   A. Many recent studies by a number of researchers have shown fairly good structure-activity correlations and some of these use molecular fragments as features. Although these were all on a small scale, they provide evidence for the large-scale feasibility provided the data can be accommodated.

   B. A small-scale study on about 200 nucleoside analogs was performed These were clustered according to structure using the University of Pennsylvania fragment set, by means of a shortest spanning path clustering method. There was a noticeable tendency of active compounds to cluster together. This confirmed results obtained by others.

2. Development of a Method

   A. The learning machine or perceptron approach has been receiving much attention lately. This approach has the attractive property that each molecule acts as a unit in the training procedure, so that correct interdependence among features is maintained. The main drawback of this method is that, if one thinks of molecules as represented by points in feature space, the outlying points have undue influence. This property is related to the peculiar requirement of a "good" ratio of training samples to features so that linear separability is likely. This method has a lengthy learning procedure but once the coefficients have been learned it is fast in evaluation.

   B. The nearest neighbor method is generally considered superior to the perceptron method since it is not unduly influenced by outlying points. However, this method would effectively require searching all the data upon each new entry and is, therefore, unappealing. To increase its reliability it is often used to see if there is activity in several, say 5 or 10, nearest neighbors, making it even more time consuming.

C. Another approach can be called Bayesian and is simply based on conditional probabilities. This approach cannot be used directly on the molecular fragment data since it requires independence of features. Gross dependencies among the molecular fragment features may be removed by trying to cluster the features and use these clusters as new features. This would still not be ideal as there will still be certain interdependencies among some of the feature clusters which would be automatically learned in the perceptron method. It turns out that there is a way to possibly get the best of both perceptron and Bayesian-type methods. This is based on a tool being developed by the principal investigator and his colleague at the Walter Reed Army Institute of Research.

Proposed Course of the Project

1. Medium scale testing of the Bayesian method as well as other basic methods will be undertaken shortly.

2. A satisfactory method will be chosen for large-scale evaluation and use.

Keyword Descriptors:

Automated classification, quantitative structure-activity relationship, structure-activity relationships, pattern recognition, feature selection, drug pre-selection, learning machines, nearest neighbor matching, Bayesian methods, molecular structure features, automated pre-selection of drugs.

MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION

INTRODUCTION

The primary function of the Mammalian Genetics and Animal Production Section is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows:

1. DR&D Screening Program.

2. Other NCI Research Contracts.

3. Research Grants (strains not available commercially and surplus animals which are offered when available.

4. Veterans Administration Research Facilities (surplus animals which are offered when available).

A secondary function of this Section is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program includes:

4 Primary Genetic Centers

5 Genetic Production Centers

5 Rodent Production Centers

18 Hybrid Production Contracts

2 Beagle Production Contracts

3 Diagnostic Contracts

2 Histocompatibility Study Contracts

2 Contracts with the National Academy of Sciences.

The Section staffing consists of one senior staff member, one professional associate, one animal contracts assistant and one clerk-typist. Also assigned to this Section is one animal caretaker supervisor and 4 animal caretakers who function in DR&D in-house laboratories.

The overall production of these facilities supplied the following strains and numbers of laboratory animals during calendar year 1974:

Mice

Inbred weanlings	1,017,484
Inbred pregnants	4,059
Inbred litters	469
Hybrid weanlings	3,545,864
Hybrid pregnants	192
Outbred mice	35,580
Outbred pregnants	289

Rats

Inbred weanlings	75,235
Inbred pregnants	46
Inbred litters	5
Outbred weanlings	14,583
Outbred pregnants	95
Outbred litters	4

Hamsters

Weanlings	25,849
Pregnants	1,293

Beagles

298

Guinea Pigs

Inbred weanlings	20

Gerbils

325

The various animal supply contracts are being restructured as indicated:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germfree state; i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed under barrier conditions.

GENETIC PRODUCTION CENTERS

Foundation animals for these centers are received from isolator colonies via germfree shippers and are placed directly into the barrier-maintained facilities for propagation. Otherwise, the same quality control standards are maintained as described for the primary centers.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed-price contractors for hybrid mouse production. The rodent production contract receives breeding stock from the genetic center expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions.

HYBRID MOUSE PRODUCTION CONTRACTS (FIXED PRICE)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts. Animals are propagated under semi-barrier conditions.

BEAGLE PRODUCTION CONTRACTS

These animals are procured on a fixed-price contract basis from qualified breeders and are supplied primarily to the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of one virus diagnostic service, one parasite diagnostic service, and one bacterial diagnostic service (primarily Salmonella and Pseudomonas species). Additionally, a limited but very significant contribution to the laboratory animal disease diagnosis program has been performed by Dr. Russell Lindsey of the University of Alabama. Dr. Lindsey has performed gross and micropathology services upon request without funding.

HISTOCOMPATIBILITY STUDY CONTRACTS

These contractors perform genetic monitoring of inbred mouse and rat strains by means of skin grafts. Additionally, one of the contracts (Health Research, Inc.) provided the program with a "hemopoietic" monitoring service whereby the source of a genetic drift which produced a degree of resistance to experimental mouse leukemias was identified.

NATIONAL ACADEMY OF SCIENCES

A small amount of support via Task Orders 6 and 16 is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines in the field of animal care and breeding. In addition, support is extended for holding conferences and meetings pertaining to the field of laboratory animal care.

DISCUSSION:

The major goal for the Mammalian Genetics and Animal Production Section is to improve the genetic purity and general health of the laboratory animals supplied to investigative laboratories.

A serious genetic problem became apparent during 1974 when it was observed that resistance to some experimental mouse leukemias (particularly L-1210) had developed in hybrid mice supplied by this Section. The source of this problem appears to have been discovered by Dr. Cudkowicz of Health Research, Inc. (Contract No. N01-CM-02035), who produced experimental evidence to the effect that a subline of DBA/2 mice, which had filtered into the breeding program of this Section, was quite resistant to normal bone marrow as well as leukemic cells from these hybrid mice. This finding was confirmed at Southern Research Institute when offspring of matings involving this subline of DBA/2 mouse were shown to be resistant to L-1210 leukemia.

It has been further established by Dr. Cudkowicz that hybrid mice (BDF_1 or CDF_1) resulting from the mating of DBA/2 males and C57BL/6 or BALB/c females do not have significant resistance to L-1210 leukemia when both parents are derived from the NIH subline. This finding has also been confirmed at Southern Research Institute by extensive testing with L-1210 leukemia implanted in BDF_1 mice originating from such matings.

Pedigreed litters from NIH sublines (DBA/2, C57BL/6 and BALB/c) are being supplied to our Section by the Small Animal Section of the Veterinary Resources Branch. Litters have been received by each of our primary genetic centers. By a strict adhesion to brother x sister matings in our foundation and expansion colonies and a continuous genetic monitoring of mice, we expect to avoid such problems in the future. The only input of breeder mice of the DBA/2, C57BL/6 and BALB/c strains of mice will be supplied by pedigreed litters from NIH on a periodic and continuing basis.

We feel that we can accomplish our other goal, the improvement of laboratory animal health, partially with the same tools that are being utilized to correct our genetic drift. The pedigreed litters that are supplied to our primary genetic centers will be brother x sister mated, after which Caesarian sections will be performed in isolators. Defined flora foundation colonies will be established under isolator conditions and expansion colonies will be maintained under strict barrier conditions. There will be no input into our primary genetic centers except under these conditions.

As in the past, animal disease problems are being temporarily controlled through a strict disease monitoring system followed by medication as indicated. In addition to the funded diagnostic centers described, we have encouraged and helped our animal suppliers to initiate a simplified system whereby they can check both incoming and outgoing animals for obvious problems.

However, we feel very strongly that the final solution to animal health problems will require the sacrificing of animals in problem areas followed by the disinfection and fumigation of rooms and areas where indicated.

To this end, we are attempting to reach a state of overproduction of some of the most needed breeder animals (DBA/2, C57BL/6, BALB/c, etc.) while maintaining other strains which are under minimal demand and/or which are not available from other sources at an acceptable level of production.

Therefore, as time and circumstances permit, we will, beginning with our primary genetic centers and moving eventually into the laboratories, gain our objective of a healthy, clean, and genetically defined animal in a clean environment with minimal animal-imposed variables for the investigator.

PUBLICATIONS SECTION

Drug Research and Development

The Publications Section directs the preparation of Cancer Chemotherapy Reports (CCR) (the principal scientific journal developed under the DCT Program), officially clears all DCT contract manuscripts, and provides editorial assistance to the DCT staff on a time-available basis. Until 1968, CCR was a single journal but in that year it was expanded to 3 parts.

Part 1: Activity in 1974

From its inception CCR (and CCR Part 1) has published original contributions in clinical and experimental studies of cancer chemotherapy, including complete works, preliminary investigations, commentaries and reviews, symposia, and letters pertinent to published articles. All candidate manuscripts are reviewed by 2 or 3 investigators familiar with the subject matter, and the final determination of acceptance or rejection is made by the Scientific Editors. CCR Part 1 is now in its 17th year of continuous publication with a total to date of 112 issues. In 1974, 6 bimonthly issues were published (total of 978 pages). A total of 191 research manuscripts were submitted to Part 1 in 1974, in addition to 53 manuscripts from symposia. This represents an increase of almost 100 manuscripts over 1973.

Part 2: Activity in 1974

CCR Part 2, which contains extensive and usually tabular studies on chemotherapy screening, structure-activity relationships, and other related subjects, has been published since 1968 as manuscripts and personnel have been available. In 1974, 4 issues were published (total of 758 pages).

Part 3: Activity in 1974

There have been 8 issues to date of CCR Part 3, which contains nonreviewable program information. In 1974, 1 issue was published (total of 87 pages). This issue contained toxicology protocal studies on a variety of drugs resulting from several DCT contract-supported institutes. A total of 21 manuscripts were submitted to Part 3 in 1974, and in the first quarter of 1975, 29 manuscripts have been received.

Comparative data on the CCR series of publications are shown in Table 1. The manuscripts accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports. The manuscripts received are reported monthly to the Associate Director, DR&D, and the Director, DCT.

New Sections in Part 1

In 1973, a section entitled Brief Reports and Preliminary Communications was added to Part 1. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work. These reports have been processed through the editorial channels more quickly than full-length manuscripts, thus reducing costs for production time and allowing more such manuscripts to reach the readers. The response to this new section has continued to be excellent during the past year. Each issue has contained 3-8 Brief Reports and an increasing number are being submitted.

In 1974, a section containing invited editorials and commentaries was initiated; several such manuscripts have been received and published. A well-known scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor. In the past year we have accepted editorials (or commentaries) on the following topics: Antiviral Chemotherapy as an Indicated Adjunct to Cancer Chemotherapy; Historical Controls Reincarnated--An Examination of Techniques for Clinical Therapeutic Research; and The Role of Structure-Activity Studies in the Design of Antitumor Agents.

Official Charter for CCR

In April 1975, the first charter meeting of the CCR Editorial Board was held. This meeting was intended to resolve several issues concerning the organization of CCR (all parts), the scope of each part, the possible simplification of the parts (ie, a primary journal and a supplement which basically serves the function of both Parts 2 and 3), a name change to reflect the new direction of the DCT, and the additional duties of the Publications Section. In addition, the Board plans to write and publish a substantive charter outlining such technicalities as the term of office for editors and the editor-in-chief, the official policy regarding review and rejection, and the specific areas of research to be covered. These joint recommendations of the Board will be submitted to the Director, DCT, for his approval and comments. In 1974, 3 new editors were added to the Editorial Board (the Board now has 6 associate editors plus the editor-in-chief). We hope to add additional members this year, especially in areas of expertise not currently covered.

Proceedings of Symposia

During the past 2 years, CCR has been approached frequently to publish the proceedings of scientific meetings. In 1974, the proceedings of 6 symposia were published, and for 1975 we have published (or will publish) the proceedings of the following 8 meetings:

1. National Prostatic Cancer Project Workshop (Buffalo, NY): June 1974
2. The Role of Metal Salts and Metal Complexes in Cancer Chemotherapy (Houston, Tex): December 1974
3. High-Dose Methotrexate Meeting (Bethesda, Md): December 1974

4. Pharmacokinetic Modeling of Anticancer Drugs (New Orleans, La): November 1974
5. Fifth New Drug Seminar on Adriamycin (Washington DC) and New Drug Symposium on Adriamycin (San Francisco): December 1974 and January 1975
6. Interim Screeners Meeting (Silver Spring, Md): May 1975
7. Plants and Cancer (Baltimore, Md): June 1975
8. Metabolism and Mechanism of Action of Cyclophosphamide (London, England): July 1975

Cumulative Index

In 1974 the editorial staff began compiling a 9-year subject and author cumulative index for CCR Part 1 (1966-1974). We hope to complete the index by late 1975 with possible publication in a supplement to the journal.

Publication Costs

Publication costs for CCR continued to remain stable in 1973 (the last year for which complete data are available). Publishing costs in this country have increased tremendously in recent years. This has naturally been reflected in higher operation costs for the Publications Section; however, the average cost per page (of Part 1) was $54.00 in 1971, $46.00 in 1972, and $45.00 in 1973. Although this is only a slight decrease, it is important in view of the increased cost of services that is occurring nationwide. We have done several things to help bring about this drop: (a) as often as possible we supply our contract printer with camera-ready art prepared by this office rather than pay for his time to produce such art; (b) we now make many corrections on the finished reproduction proofs instead of requesting the printer to make such changes; and (c) the new Brief Reports are, by design, short and uncomplicated and therefore require less work by the typesetter. In addition, our new printing contract for 1975 requires the typesetter to use a slightly smaller typeface, thus allowing more type to be printed on each page. Even a small reduction in pages will help to reduce paper costs.

Coverage of CCR in Current Contents and Related Publications

Since 1967 CCR has been listed in Current Contents, Life Sciences. In 1973, CCR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CCR began sending corrected page proof to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973 CCR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes the Index to the Periodicals of the US Government. This trend toward more extensive coverage of CCR indicates a growing interest in the field and in CCR in particular.

Outside Evaluations of CCR

In 1974 CCR was evaluated by Informatics Inc., an information systems company, as part of a contract with the Office of the Director, NCI. This evaluation was done by interviewing subscribers to CCR over the phone and by mail. The overall assessment of CCR was favorable: "over 78% of the users of CCR favor its publication very strongly, while 19% favor its continuation moderately." The final report included 11 specific recommendations for CCR including (a) a modification of the current Parts, (b) publication of a cumulative index, (c) inclusion of immunotherapy in the subject scope of the journal, and (d) expansion of the Editorial Board. We have attempted to discuss each point raised by Informatics, and in most cases steps were taken to respond to the suggestions. This survey will be especially useful in 1976 when we will submit our next request for funding to the Office of Management and Budget.

In 1974 another analysis of the journal was obtained from the Institute for Scientific Information. The analysis compared CCR with 12 other cancer journals. For the years 1969 and 1972 we received a ranking according to: (a) number of times CCR was cited in references, (b) the impact factor (measure of average number of citations received by each source item published in the journal), and (c) immediacy impact (measure of rapidity with which articles in the journal become cited). The evaluation showed that CCR remained stable with regard to the number of times it was cited, but that the impact factor rose from 8th place to 4th place (among the 13 journals), and the immediacy factor rose from 10th place to 6th place. We feel that these changes are significant, especially since the publication time has been reduced further since the period covered in the survey. We plan to have this inexpensive and valuable survey taken each year.

Office of Management and Budget Approval

In late 1973 we submitted to the OMB our request for continuation of funds. This document outlined the purpose and scope of CCR, the publication costs incurred by the staff, a breakdown of the distribution of each issue (including the categories of subscribers), and the justification for continuing the publication. In early 1974 we received OMB approval which allows us to continue production through November 1976, at which time we will be required to request further funding.

Distribution of CCR

The Publications Section is also responsible for maintaining the free mailing list for CCR. CCR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3700	Foreign and domestic free mailing (includes ca 175 at NIH plus 225 storage copies for Section)
1200	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depositiory Libraries)
5267	

In the past several years the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of a recent limit (3300 names) established by the OMB on the number of free subscriptions. We currently have over 100 names on a waiting list to be added to the free list, and several hundred requests have been forwarded to GPO for paid subscriptions. The demand for specific back issues remains high.

Problems with the service rendered by the GPO in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in 1973 the mailing list was transferred to the Distribution Section, Printing and Reproduction Branch, ODA, NIH. This conversion has allowed more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing tapes to be used by the contract printer in mailing the issues to the subscribers. NIH has been able to perform these functions in one fourth of the time GPO previously required. In 1975 we began a screening of the mailing list to eliminate addresses of subscribers who have moved, retired, or died. This clearing of the mailing list will probably allow us to add most of the names on our waiting list.

Contract Manuscripts Clearance

Another function of the Section is the clearance of DCT contract manuscripts for publication. In 1974, 184 of these manuscripts were cleared, and during the first quarter of 1975, 71 manuscripts were cleared. In assuming the responsibility for clearance of these manuscripts, the Publications Section checks all NSC numbers mentioned in the papers and notifies the DDB when compounds must have publication clearance from suppliers. The authors are instructed to reword footnotes as necessary to give proper credit to the DCT. The Section ensures that the Project Officer sees all papers and signs the correct forms. Records and reprints are kept for all manuscripts cleared, and quarterly reports are prepared for distribution to staff personnel.

Table 1

Year	Part	Mss rec'd	Mss accepted	Issues published	Pages published	Reviewers	Total printing costs ($)	Cost per page ($)
1970	1	122	68	6	492	140	15,334	31.16
	2	5	4	--	--			
	3	1	--	--	--			
		128	72					
1971	1	107	73	5	621	164	33,284	53.59
	2	6	5	1	246		14,828	60.27
	3	11	11	1	104		4,564	43.88
		124	89	7	971		52,676	
1972	1	122	76	6	814	197	37,715	46.33
	2	4	5	1	353		21,064	59.66
	3	47	47	2	181		10,863	60.01
		173	128	9	1348		69,642	
1973	1	148	112	4	524	167	23,713	45.25
	2	19	19	--	--			
	3	77	76	3	576		26,184	45.44
		244	207	7	1100		59,897	
1974	1	244	138	6	978	212	40,000	
	2	21	21	4	758		30,000	
	3	21	21	1	87		4,000	
		286	180	11	1823			
1975 (Jan 1- Apr 9)	1	64	44	2 (in press)	456	157	Not available	
	2	--	--	--				
	3	29	29	(1 in press)				
				11 planned	1850 planned			

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

As the national and international effort on the eradication of cancer is accelerated, the expanded research, development and application will result. The major avenues for cancer treatment presently being followed are surgery, radiation and chemotherapy, and to a lesser extent, immunotherapy. Optimal single modality regimens are to be integrated into combined modality approaches in the treatment of local and regional and disseminated disease where appropriate. Since different cancers have different therapeutic requirements the treatment approach must be disease oriented rather than modality oriented. One avenue of the treatment approach is via chemotherapy. The foundation of chemotherapy is the drug itself - either single or in drug combination. Consequently, the search for more effective, less toxic and broader acting agents will continue.

Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetcs observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information accumulates and greater achievement is being experienced in the control of the more rapidly proliferating phase of cell growth or young tumors, the search for new structures aimed against the slower growing tumors as in specific areas, i.e., lung, breast, large bowel, pancreas, kidney, etc. will be increased.

Objectives

As part of the broad Division of Cancer Treatment Program, the Drug Development Branch has three major objectives:

I. Fundamental Chemical Research

   a. chemical synthesis of new compounds;

   b. extraction, isolation and identification of new active agents from natural sources;

   c. theoretical design of new chemical structures via structure-activity evaluations in collaboration with investigators of biochemistry, biology, toxicology, pharmacokinetics, and clinical trials; and

   d. development of methodology for drug assay.

II. Worldwide Surveillance

   a. information on potential antitumor chemicals and drugs; and

   b. discovery sources of new chemicals and drugs for biological evaluation.

III. Applied Services

   a. accessioning and chemical nomenclature of materials entering the program;

   b. development, maintenance and utilization of a computer data bank for chemical and drug information;

   c. correlation of chemical structure to biological activity;

   d. chemical resynthesis and production of active agents;

   e. quality control and analytical evaluation of drugs and bulk chemicals for clinical and preclinical toxicological evaluations;

   f. chemical synthesis of active radiolabeled drugs;

   g. development and production of suitable dose formulations for use in clinical studies; and

   h. storage and distribution of bulk chemicals and clinical dose formulations.

Implementation of Program

These objectives are carried out through the activities of the Branch's seven Sections which are:

I. Chemical and Drug Procurement Section: The objectives are divided into the synthesis, preparative, and quality control activities. The synthesis activites are involved with the synthesis of new compounds of diverse structures for antitumor evaluation. As the rapidly growing tumors become more and more under control, attention will be focused upon developing new drugs for the slower growing tumors, especially for lung, breast, colon and pancreas.

The preparative activities include the process development and improvement of procedures for large scale production; the synthesis and isolation of large quantities of bulk chemicals and drugs for confirmational testing, pharmacological and toxicological evaluation, and clinical studies; and preparation of high purity radiolabeled materials for the chemotherapy studies. In addition, the procurement via purchase of large quantities of certain bulk chemicals and drugs needed by the Program is included in the preparative activities.

Chemical and quality control-analytical data are provided to the Food and Drug Administration (FDA) for the filing of Investigational New Drug (IND) applications.

II. Natural Products Section: The objective is the development of novel drugs from: 1) higher plants and animal (marine and insect) sources and 2) microbial sources. In support of these tasks, the Section maintains a worldwide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, the isolation and identification of the active components; and in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clinical studies. Also, the Section directs a program for the investigation of three groups of organisms, the bacteria, the fungi, and the actinomycetes. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

III. Drug Design and Chemistry Section: The objectives of the intramural laboratory effort are: 1) chemical research in the design and synthesis of new drugs for the treatment of cancer, 2) the chemistry of agents and drugs of interest in the treatment of cancer, 3) investigation of the chemistry of natural products as potential antitumor drugs, research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, and 4) the correlation of structure-activity relationships to summarize existing data and to provide guidelines for analog synthesis.

Individual Progress Reports (Serial Nos. Z01 CM 03580-06 DD, Z01 CM 03581-06 DD, Z01 CM 035282-01 DD) are presented later in this report.

IV. Clinical Drug Distribution Section: The objectives of this Section are to provide the Program with suitable formulated drugs for clinical evaluation. The principal activities include development of experimental dosage forms, production of various dosage forms for clinical investigative trial, surveillance of the quality of such dosage forms, procurement of commercially available pharmaceutical products, and storage and distribution of the clinical products used in the Cancer Treatment Program. In addition, the Section provides the necessary supportive data for the filing of IND applications with the FDA.

V. Chemical and Drug Records Section: The objectives are to 1) assist in the worldwide surveillance and collection of new drugs and provide the program with possible sources of such agents, 2) manage the acquisition of chemicals contributed to the Program, 3) develop, maintain, and utilize a chemical data bank on materials of use to the program, 4) develop structure-activity methodology for new materials to be synthesized in collaboration with the Chemical and Drug Procurement Section and the Drug Design and Chemistry Section, 5) maintain adequate storage and inventory of bulk chemicals, and 6) coordinate the distribution of materials to screeners and other investigators. Surveillance is also provided over industrial 'Commercial Discreet' products.

VI. Data Processing and Computer Systems Development Section: The objectives of the Section are to develop data processing systems to meet the scientific, operational control, and management planning needs of the Branch. The Section is primarily concerned with, 1) directing contract effort to implement a new automated Chemical Information System (CIS) which supports such activities as accessioning, storage, and distribution of compounds acquired for antitumor testing; 2) directing and coordinating the activities of the contractor responsible for operating the Chemical Information Systems. In addition, the Section is engaged in pursuing a project to improve the utilization of automated literature and chemical files maintained by Federal agencies and industrial organizations to acquire compounds of interest to the program.

VII. Scientific Records Section: The objectives are to coordinate information on all new drugs acquired for testing in a manner that allows fast processing prior to biological evaluation and facilitates retrieval from the chemical file retrospectively by individual compound or by chemical class using manual, microfilm and computer system. In addition, the Section personnel, 1) prepares or monitors the preparation of all chemical input in a standard format for duplicate checking, 2) maintains the master chemistry card files and suppliers addresses, 3) supervises the maintenance of computer name and formula indexes to the file, 4) prepares documents for the shipment of all new acquisitions, 5) provides retrospective searching of the file using the index and substructure search programs, and 6) investigates new methods to facilitate chemical information retrieval and dissemination.

Branch Staff

The Drug Development is composed of a staff of forty-six persons consisting of eight senior professional scientists (GS-13 and above), eleven other professional staff members (GS-9 to GS-12), three PHS Commissioned Corps Officers, one Staff Fellow, six Visiting Fellows and the remainder (17) technical and clerical staff.

Those responsible for the activity of the seven Sections are as follows:

Drug Development Branch
Chief -- Dr. Harry B. Wood, Jr.

Chemical and Drug Procurement Section
Head -- Dr. Robert R. Engle

Natural Products Section
Head -- Dr. Jonathan L. Hartwell (retired 3/1/75)
Acting Head -- Dr. John D. Douros

Drug Design and Chemistry Section
Head -- Dr. John S. Driscoll

Clinical Drug Distribution Section
Head -- Mr. J. Paul Davignon

Chemical and Drug Records Section
Head -- Mr. Robert B. Ing

Data Processing and Computer Systems Development Section
Head -- Mr. Sidney Richman

Scientific Records Section
Head -- Mr. George F. Hazard

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section.

Major Branch Highlights

In addition to the Branch accomplishments listed later in the report, the major highlights are listed here:

1. Methotrexate: The heightened interest in Methotrexate, because of its demonstrated utility in high dose regimens for the treatment of osteogenic sarcomas and other cancers following surgery, has placed a demand for both larger quantities and higher quality of the drug. The Drug Development Branch, through its contract at Southern Research Institute, has achieved a breakthrough by developing a new, improved synthetic procedure. The new method is rapid, relatively simple, and provides the drug in high purity at a lower cost. The method is being utilized successfully for large scale production by other NCI contracts. Patent application has been filed.

2. Vincristine: Basic chemical modifications and new analog development on this complex alkaloid have been lacking due to the complex chemical nature of this compound. A major breakthrough has been made by our contractor at the University of British Columbia. A synthetic procedure which provides the correct stereo-chemistry at the bridging carbons of

the dimeric halves of the vinca alkaloids has been discovered. This breakthrough will provide both vincristine and analogs with the natural chemical configuration. A patent application has been filed.

3. Daunomycin: A new source of this antibiotic has been developed at Parke Davis & Company which can provide multi-kilogram quantities of high quality material at a very modest cost.

4. New Suppliers: Three hundred and twenty eight new suppliers, of which 64 were industrial and 264 were non-industrial suppliers, have contributed new agents for evaluation in the cancer program. From all sources 48,370 new materials were received for evaluation.

5. Fort Detrick: The research laboratories at Frederick Cancer Research Center have been completed. The pilot plant design and construction are scheduled for completion in 1975. The major purpose of the fermentation research program is to isolate unique organisms and ferment them under varied conditions with rare types of media, hopefully to produce novel antineoplastic agents. The purpose of the pilot plant is to develop processes and produce antineoplastic agents which are uncovered by the entire natural products program.

Operation of Program

The methods for selecting new agents for input into the primary screen is gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. This should result in more active analogs of existing active materials, fewer biologic tests required, and logically, the number of active agents should be higher. Acquiring such preselected agents may be more costly. Changes in the screening protocol should be incorporated to include differentiation of analog activity, including toxicological evaluation where appropriate.

During 1974 a large number of exploratory compounds were contributed from random sources but effort will be directed toward acquiring rationally designed compounds. Unfortunately, the state-of-the-art has not reached a degree of sophistication which allows a completely rational approach to drug design. However, as preselection increases and as the structure-activity mosaic gradually takes shape, the choice of chemical compound for introduction into the screen will emerge more from direct synthesis and will gradually replace random procurement. The gradual mutation from random input to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

One major thrust of analog synthesis will be in the area of clinically effective drugs. Analogs will be designed for therapy improvement and decreasing the undesirable features of the parent drug. Also analog synthesis will be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. Synthetic analogs of known active drugs may possess superior properties because of better transport and greater resistance to degradation or greater binding to appropriate enzyme sites. Synthetic analogs also may produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modification could also introduce new chromophore groups to aid in determining by analysis the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of whole body drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical cell cycle kinetic studies, pharmacological and pharmaceutical rationales, and biological activities demonstrated in non-cancer programs, should provide additional input to the directed preselection and synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biologic and/or biochemical screens.

In addition to the search for 'mitotically linked drugs', directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which have a high degree of organ localization will be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood-brain-barrier. A program has been developed to synthesize CNS active antitumor agents. Also, drugs localizing in the lung, breast, large bowel, bladder, etc. will be considered in modifying their chemical structure with the objective of introducing antitumor properties. Organ specific screens are needed to evaluate such new types of agents. The increasing awareness that many antitumor drugs may possess latent carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of large amounts of drugs for the clinic.

The Branch is continuing its search for natural products, which often provides an invaluable source of medicinal agents and new chemical structures. Plants continue to provide many extracts with significant tumor-inhibitory activity. It is important that the compounds responsible for this activity be isolated and characterized. Some will be useful in chemotherapy, others will be useful as tools in elucidating selective growth-regulating mechanism or as templates for syntheses of more effective agents.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives for the purpose of defining the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements which are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

Seminars

The Branch sponsored the following seminars:

Structure-Activity Relationships in the Acridylmethanesulfonanilide-AMSA-Series by Dr. Bruce F. Cain, Auckland Division Cancer Society of New Zealand, Auckland, New Zealand.

Biologically Active Compounds Isolated from Fermentation Products by Professor Wolfgang Loeffler, Institut fur Biologie II der Universitat Auf der Morgenstelle, Germany.

Herbal Medicine in China by Professor Norman R. Farnsworth, University of Illinois, Chicago, Illinois.

Customized Cancer Therapy by Dr. Seymor Gelfant, University of Georgia Medical School, Augusta, Georgia.

Studies Related to the Carrier Principle in the Design of Cytotoxic Drugs by Dr. Maria Szerke, IREX Visiting Scholar, Eotvos L. University, Budapest, Hungary.

Approaches to Ion Structure: Ion Kinetic Energy Spectrometry by Dr. Michael K. Hoffman, Kansas State University, Manhattan, Kansas.

Mass Spectrometry of Metabolites by Dr. Stephen Billets, University of Mississippi, Oxford, Mississippi.

Symposia

A Cell Growth Regulators Symposium was held in September and was attended by 26 scientists. The objective of the symposium was to transmit information to the NCI group on the potential of cell growth regulators in cancer control. In addition, the symposium updated the attendees on the newest theories and experimental research being done in the field of cell growth regulators. The presentation focused on the fact that there are different levels of mitotic control operating throughout the body.

Active Participants:

Dr. I. Cameron	The University of Texas
Dr. B. Lozzio	The University of Tennessee Memorial Research Center
Dr. F. Hauser	Oregon Graduate Center for Study & Research
Dr. S. Gelfant	Medical College of Georgia

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data must ever be present. A total of 3132 individual suppliers, of which 1308 are industrial suppliers, have contributed samples to the Program. During 1974, there have been 264 new non-industrial suppliers and 64 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many materials have been supplied from foreign countries. The increasing number of contributors is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for the acquiring new agents, synthetic and natural products, has provided over 560 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to NCI and the scientific community-at-large. International visits were made by the Drug Development Branch staff during this period to the following:

Dr. Wood

Drs. T. C. Loo (Director), M. L. King, C. Y. Choi, Y. E. Wang, K. Y. Cheng and C. C. Chiang, National Defense Medical Center, Taipei, Taiwan.

Dr. Wang Chi-wu, National Science Council, Taipei, Taiwan.

Drs. Wood and Douros

Drs. Yoshio Sakurai, S. Tsukagoshi, Cancer Chemotherapy Center, Tokyo, Japan.

Dr. H. Umezawa, Microbial Research Foundation, Tokyo, Japan.

Dr. Y. Yagisawa, Japan Antibiotics Research Association, Tokyo, Japan.

Drs. T. Nara, T. Hirata, T. Kagawa, S. Itagaki, H. Samejima, S. Ishikawa and K. Arakawa, S. Kinoshita, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan.

Drs. M. Arakawa, M. Konishi, H. Okazaki, M. Arai, and Tsuyoshi-Iida, Sankyo Co., Ltd., Tokyo, Japan.

Dr. M. Aihara and Y. Hiraska, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

Drs. Taymia, A. Kobayashi, K. Yamashita, T. Oritani and K. Matsuda, Tohoku University, Sendai, Japan.

Drs. K. Ogata and Y. Tara, University of Kyoto, Kyoto, Japan.

Dr. Sugino, Y. Kadota, Y. Kuwada, Z. Suzuoki, T. Kishi, and T. Matsumoto, Takeda Chemical Industries, Ltd., Osaka, Japan.

Drs. T. Marumo, H. Kaneko, Y. Minamiura, and Yokagawa, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan.

Drs. T. Okabayashi, H. Minato, K. Katagiri, T. Ishigami, Z. Suzoki and A. Takamizawa, Shionogi Research Laboratories, Osaka, Japan.

Dr. Douros

Dr. K. Arai, Chiba University, Chiba, Japan.

Dr. H. Maeda, Kumamoto University, Kumamoto, Japan.

Dr. K. Matsumoto, Mitsubushi Chemical Industries Ltd., Tokyo, Japan.

Dr. Engle

Dr. R. J. Bridgwater, Maybridge Chemical Company, Ltd., Trevillett, Tintagel, England.

Drs. G. Hug, W. Hanske, R. Freudenthal, Heinrich Mack Nachf, Illertissen, West Germany.

Dr. G. Zobel, Alcor Chemie A.G., Mettmenstillen, Switzerland.

Accomplishments

The Drug Development Branch provided a steady flow of materials into the Program. These materials consisted of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point II). Three Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table I). Of the 39,814 new crystalline materials added in 1974 to the Program, 1153 compounds (2.9%) were acquired under contract, 4121 compounds (10.4%) were purchased, 26,254 compounds (65.9%) were voluntarily submitted from industrial sources, 8286 compounds (20.8%) were from non-industrial suppliers.

The Branch manages a total of 53 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 11 million dollars (FY-75) and covering the major areas as shown under the primary responsibilities of the Branch. Of the 53 contracts, 50 are domestic and 3 are foreign contracts.

In summary, the following were achieved during the calendar year of 1974 by the Drug Development Branch:

1. A total of 48380 new substances (Table I) was obtained for screening or other evaluation;
    a. 39540 synthetics
    b. 725 antibiotic cultures
    c. 5830 plant materials
    d. 2011 animal materials
    e. 274 other crystalline materials
2. A total of 1370 compounds were prepared under contract;
    a. 217 were resynthesized by the preparation laboratory contracts
    b. 1091 were synthesized as new compounds from synthesis and prep lab contracts.
    c. 62 were from the natural product laboratory contracts.
3. Seven new substances were assigned to Decision Point II (Table I and II) (candidates for preclinical toxicology);
    a. 4 fermentation products
    b. 3 synthetic compounds
4. Three drugs were assigned IND numbers (Table I and III)
    a. 2 fermentation products

   - b. 0 plant product
   - c. 1 synthetic compound

5. Nine hundred fifty-one compounds were assigned to Step III (Table I);

6. Analytical-Quality control determinations were made on 225 different lots of 90 different compounds representing 157 lots of bulk compounds and 68 lots of clinical formulations;

7. Twelve radiolabeled compounds (Table IX) were procured;

8. Three new dosage forms were developed and 13 existing dosage forms were modified;

9. Approximately 7,554,533 formulated units were procured;
   - a. 5,038,270 compressed tablets
   - b. 522,750 hard gelatin capsules
   - c. 1,993,513 vials

10. Fifty-three contracts were supervised by the Branch;
    - a. 16 synthesis laboratories
    - b. 8 preparation laboratories
    - c. 9 plant and animal products
    - d. 5 drug formulations
    - e. 5 fermentation products
    - f. 2 analytical-quality control
    - g. 2 radiolabel laboratories
    - h. 6 miscellaneous

11. There were 180 Grant Proposals and 3 Patent Applications reviewed and commented upon;

12. Samples were received from 64 new industrial concerns and 264 new non-industrial sources;

13. Fifty-six manuscripts by contractors were cleared for publication and nine manuscripts were published by the Branch staff;

14. One scientific symposia and 8 seminars were sponsored.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT(1)

	CRUDE EXTRACTS				CRYSTALLINE MATERIAL SUBMITTED		STEP III(2)		DECISION POINT II(3)		IND FILED	
	NO. SUBMITTED		NO. ACTIVE									
	1973	1974	1973	1974	1973	1974	1973	1974	1973	1974	1973	1974
Fermentation Products	2550	725	267	174	136	94	17	15	--	4	2	2(4)
Animal Products	2698	2011	83	221	1	76	--	--	--	--	--	--
Plant Products	11661	5830	222	549	154	104	7	10	1	--	1	--
Synthetics	--	--	--	--	34143	39540	907	926	9	3	11	1
Total	16900	8566	572	944	34434	39814	931	951	10	7	14	3

(1) Based on Calendar year 1974

(2) Showing Confirmed Activity

(3) Selected Candidates for Preclinical Toxicological Evaluation and Clinical Trial

(4) Vaccines

TABLE II

COMPOUNDS PASSING DECISION NETWORK II A

NSC NO.	COMPOUND NAME	SOURCE
143,095	Pyrazole-3-carboxamide, 4-hydroxy-5-β-*D*-ribofuranosyl- (Pyrazomycin) (Pyrazofurin)	Fermentation
157,365	Neocarzinostatin	Fermentation
163,501	5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro- (AT-125)	Fermentation
170,105	Macromomycin	Fermentation
172,755	Glycine, *N*-[1-oxo-5-(1*H*-Purin-6-ylthio)pentyl]-, ethyl ester (Butocin)	Synthetic
176,319	Quinolinium, 6-amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]phenyl]amino]carbonyl]phenyl]amino]-, dibromide	Synthetic
178,248	D-Glucopyranose, 2[[[(2-chloroethyl)-nitrosoamino]carbonyl]amino]-2-deoxy- (chlorozotocin)	Synthetic

TABLE III

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
132313	Galactitol, 1,2,5,6-dianhydro-	Synthetic
220537	Corynebacterium Parvum Vaccine	Fermentation
B-116327	Mycobacterium Tuberculosis Vaccine (BCG) (Connaught)	Fermentation

Publications By Contractors

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CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to synthesize new compounds for antitumor evaluation; to procure large quantities for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trial in humans; to procure radiolabeled materials; and to evaluate the materials as to their identity, assay and purity.

The work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;
2. The Preparative Activities, which synthesize and isolates (a) bulk chemicals and drugs and (b) radiolabeled materials for toxicological, pharmacological and clinical investigations, for confirmational testing and for completion of primary evaluation; and
3. The Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the toxicological, pharmacological and clinical programs. Solubility, stability, and cost data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section consists of four professional staff and a secretary. One professional person is involved full time with the quality control activities. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;
2. Biologically active natural occuring compounds;
3. Leads generating from the screening program;
4. Biological and biochemical rationale;

5. Leads from the literature;

6. Compounds to explore structure-activity relationships;

7. New structural types; and

8. Agents to resolve questions on chemical and biochemical mechanism of action.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited by severe toxic side effects and/or are associated with the development of drug resistant tumors. In addition, many tumors do not respond to any of the current drugs. Therefore, a greater variety of agents with diverse structures and mechanism of action, broader tumor spectrum, and greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. The following are some examples:

1. A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources and/or are very expensive, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

   The clinically effective antibiotics adriamycin and daunorubicin are obtained in rather poor yields from the natural source. These compounds have dose limiting toxicological (cardiac) properties and, therefore, the synthetic effort at Stanford Research Institute towards developing a practical total synthesis of the antibiotics and on the preparation of analogs and derivatives has continued. Reasonable progress has been made towards the total synthesis of adriamycin. Twenty derivatives of adriamycin and daunomycin have been prepared including a series of rubidazone analogs, carbonyl, and amino derivatives. Some semisynthetic and totally synthetic analogs have been prepared and submitted for biological evaluation. The work in this area is continuing and it is anticipated that the effort in this very interesting area will be increased during the coming year.

   Some synthetic effort has been and is being devoted to the development of a general and versatile synthesis of the highly complex but clinically effective dimeric Vinca alkaloids, vincristine and vinblastine. The synthesis of related analogs and derivatives is also being undertaken. These analogs and derivatives should provide information concerning the mechanism of action, their metabolism and structure-activity relationships. Also, a synthesis of these materials would provide the means of obtaining radiolabeled vincristine, vinblastine, and related dimeric alkaloids for metabolism and drug distribution studies. The Vinca alkaloids, from the plant Vinca rosea L, are obtained via a very complex and costly extraction procedure. The compounds are present in only minute quantities in the plant. The development of a synthesis for these materials has the potential of providing the means of obtaining analogs, and of making these materials available in larger quantities and at a lower cost. This work is being done at the University of British Columbia and the effort is subdivided into the following areas: (1) studies of the dimerization reaction and synthesis of dimeric compounds for biological evaluation; (2) development of novel separation and analytical techniques for use in synthetic and metabolic studies; (3) partial synthesis of appropriately functionalized indole units; and (4) partial synthesis of dihydroindole units. Considerable progress has been made in all the areas and a major breakthrough has been made in the synthesis of novel synthetic dimers. A synthetic procedure which provides the correct (natural) stereochemistry at C_{18} (the point of attachment of the indole and dihydroindole units) has been developed. This procedure provides the means to obtain dimeric analogs with the desired chirality at C_{18} as well as the naturally occurring alkaloids via synthesis.

A limited amount of synthetic effort is devoted to the total synthesis of the antitumor antibiotic Belomycin. This complex antibiotic has been divided into five unique units and the synthesis of each unit is being undertaken. After completion of the synthesis of the five units they will be combined to complete the total synthesis. Several key intermediates required for the synthesis of three of the five units have been prepared and sample of these key intermediates have been submitted for screening. This work is being undertaken at Massachusetts Institute of Technology.

The antibiotic Streptonigrin has demonstrated a high degree of antiviral activity in addition to antitumor activity and is obtained in rather poor yields from the natural source. Some effort is being devoted to developing a total synthesis of this material, which will also provide new analogs. This work is being done at Midwest Research Institute and is continuing.

A number of compounds related to the antileukemic alkaloids coralyne and nitidine have been prepared at Midwest Research Institute utilizing the procedure recently completed by this group for the total synthesis of these alkaloids. The compounds have been submitted for antitumor evaluation and some structure-activity correlations with the compounds of these groups have been undertaken.

A limited amount of effort has been devoted to improving technique for the separation of the streptovaricin complex and a number of minor components has been isolated and characterized. In addition the preparation of some derivatives of the streptovaricins and geldanamycins have been prepared and evaluated as inhibitors of reverse transcriptase. This work was done by the University of Illinois.

Some effort is being extended to the preparation of some analogs and derivatives of the interesting pyrrolizidine alkaloid, indicine N-oxide and the biochemically interesting antibiotic, sparsomycin. The synthetic analogs are being prepared in an attempt to obtain more active derivatives and to determine the structural parameters for activity. This work is being done by Microbiological Associates, Inc.

2. Some of the synthetic effort has been devoted to the preparation of analogs and derivatives of folic acid. The major effort has been directed towards developing new and improved methodology for the preparation of compounds of this type. A major breakthrough has been made in this area by investigators at Southern Research Institute. A new and improved procedure for the preparation of the clinically effective antifolate, Methotrexate, has been developed and the large scale production of this agent utilizing this new method is in progress. Several sidechain altered variants of Methotrexate were prepared and the preparation of other folic acid derivatives utilizing the newly developed method is in progress. Improved methods for the preparation of citrovorum factor are also being investigated.

3. The area of nucleosides has already provided a number of very interesting compounds with antitumor activity. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to undesirable enzymatic degradation in the cancer patient.

   A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute, Stanford Research Institute, and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) tricyclic nucleosides, (c) nucleosides of pyrazolo-[3,4-*d*]pyrimidine, pyrazolo[3,4-*b*]pyridine, imidazo[4,5-*c*]pyridine and imidazo[4,5-*b*]pyridine, (d) imidazole nucleosides, (e) adenosylmethionine analogs, (f) "Carbocyclic sugar" nucleoside derivatives, (g) "carbon-carbon nucleosides", (h) deazapurine nucleosides, (i) 2 and 8 azapurine nucleosides, (j) "transition-state" nucleosides, and (k) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars. The work in this area is continuing.

4. Some effort has been devoted to the isolation and synthesis of metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation *in vivo*. Work in this area is continuing at Collaborative Research, Inc., and Southern Research Institute in an effort to determine the mechanism of activation of this active agent and to isolate and synthesize the active metabolite(s).

5. In reviewing the compounds which have shown activity in the program the largest majority of the compounds have contained nitrogen and many have been nitrogen heterocycles. Some of the synthetic effort is being devoted to the synthesis of new nitrogen heterocycles as potential antitumor agents. The emphasis in this area has been on the preparation of imidazo[1,5-a] pyrazine, 1- and 2-N-oxides of trisubstituted-1-pyrazoline, 1,2,3-triazole-2-oxide, triazepine, azirine, tetrazene, and oxathiazene derivatives. Several compounds of these types have been prepared and submitted for evaluation by the University of Colorado, the University of Michigan, and the University of Rhode Island.

6. The class of compounds known as N-nitrosoureas have demonstrated a high degree of antitumor activity and some possess the ability to cross the blood-brain barrier. Three compounds of this class (BCNU, CCNU, and Methyl CCNU), are of prime clinical interest. A few additional examples of this structural type have been synthesized by Southern Research Institute with emphasis on analogs of Methyl CCNU for improved solid tumor activity, analogs of chlorozotocin for reduced bone marrow toxicity, and metabolites of CCNU.

7. The design and synthesis of oligo and polynucleotides as potential inhibitors of the RNA dependent DNA polymerases and other polymerases are under investigation. Some oligoribonucleotide primers and some modified templates were prepared by Collaborative Research, Inc.

   In addition, some synthetic effort has been devoted to the synthesis of some derivatives of 3-formylrifamycin as potential polymerase inhibitors. Also, some ansa bridge modified rifamycins have been prepared and submitted for evaluation. Some of the rifamycin derivatives prepared were found to be selective inhibitors of RNA dependent DNA polymerase as well as inhibitors of focus formation. This work is being done by Dow Chemical Company.

8. Some of the synthetic effort of the Section has been directed towards the synthesis of compounds which potentially may localize in specific organs or parts of the body. The main effort in this area during this report period has been to develop CNS active antitumor agents. Some derivatives of ergocornine, a CNS active natural product in the lysergic acid family, have been prepared. This work is being done at Sheehan Institute for Research.

9. In order to extend the discovery of the high degree of antitumor activity in the coordination compound cis-diamminedichloroplatinum which is currently being studied clinically, the preparation of some coordination compounds of some of the transition metals has been continued. Coordination compounds of platinum, rhodium, niobium, cobalt, copper and nickel have been prepared and are being evaluated in the standard tumor test systems. The Pennwalt Corporation is investigating this area.

10. Some of the synthetic effort of the Section has been directed towards the synthesis of Procarbazine analogs and related methylhydrazine derivatives. Several compounds of this structural type have been prepared and submitted

for evaluation by Midwest Research Institute.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Cancer Treatment Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure. During the past year, seven new compounds passed Decision Point II, of which three were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs 16 contracts for the chemical synthesis of new compounds for the Program (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR SYNTHESIS

CONTRACT	INVESTIGATOR	CONTRACT NO.
British Columbia, University of	Kutney	N01-CM-23223
Collaborative Research, Inc.	Friedman	N01-CM-60506
Collaborative Research, Inc.	Friedman	N01-CM-12326
Colorado, University of	Hassner	N01-CM-43779
Dow Chemical Company	McCarthy	N01-CM-33724
Illinois, University of	Rinehart	N01-CM-23208
Massachusetts Institute of Technology	Hecht	N01-CM-43712
Michigan, University of	Burckhalter	N01-CM-92004
Microbiological Associates, Inc.	Beisler/Dubois	N01-CM-43761
Midwest Research Institute	Cheng	N01-CM-33743
Pennwalt Corporation	Block	N01-CM-12309
Rhode Island, University of	Goodman/Abushanab	N01-CM-43778
Sheehan Institute for Research	Razdan	N01-CM-23719

CONTRACT	INVESTIGATOR	CONTRACT NO.
Southern Research Institute	Montgomery	N01-CM-43762
Stanford Research Institute	Henry	N01-CM-33742
Utah, University of	Townsend	N01-CM-43806

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quantities needed from the original supplier or on the open market. These laboratories differ from the synthesis laboratories in that they are service contracts for resynthesis of known chemicals and for the large scale extraction, isolation, and purification of materials from natural sources.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies, and cost data are also provided by these contractors. These contractors have, on occasion, provided material in formulated dose form.

The increased emphasis in clinical investigations has caused a major increase of the preparation activities. An increase in the number of compounds demonstrating confirmed activity will result in an increase in the number of compounds to be resynthesized. Also, the increased effort being devoted to toxicological and clinical studies has resulted in the need for even more compounds from this program and in larger quantities.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemical and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons: (a) clinical investigations; (b) preclinical toxicological and pharmacological evaluation; (c) maintaining a stock of chemicals of clinical and preclinical interest; (d) completion of screening or confirmational testing; and (e) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to 200 kilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 217 compounds totaling 161 kilograms during the calendar year 1974. Included in this group were 38 compounds scheduled for or undergoing clinical evaluation totaling 157 kilograms. In addition to the compounds resynthesized, 116 new compounds were also prepared by the preparative laboratories. The Section also purchased 17 compounds scheduled for or undergoing clinical evaluation totaling 359 kilograms.

The Section directed eight contract laboratories for the preparation of materials needed by the Program (Table V).

TABLE V

CONTRACT LABORATORIES FOR PREPARATION OF CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Aerojet Chemical Company	Jagow	N01-CM-81300
Aldrich Chemical Company	Li	N01-CM-23706
Dow Chemical Company	Collins	N01-CM-12165
Merck and Company	Babson	N01-CM-22002
Monsanto Research Corporation	Ellard	N01-CM-33709
Parke, Davis & Company	Elslager	N01-CM-23708
Pharm-Eco Laboratories, Inc.	Draper	N01-CM-23707
Starks Associates, Inc.	Starks	N01-CM-23203

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. A total of 12 radiolabeled materials (Table VI) have been procured from all sources. Ninety-five shipments of radio-active substances were made during calendar year 1974.

TABLE VI

RADIOLABELED MATERIALS PROCURED

NSC NO.	RADIOLABELED MATERIALS	LABEL
740	Methotrexate	H^3(3',5')
4728	1,3,4-Thiadiazole,-2-amine	C^{14}(5)
63878	Cytosine, 1-β-D-arabinofuranosyl- (ara-C)	H^3
77037	Tetrandrine	C^{14} (methyl)
77213	Procarbazine	C^{14} (N-methyl)
102627	3,3'-Iminodipropyl dimethanesulfonate hydrochloride	C^{14} (propylene)
102816	5-Azacytidine	C^{14} (4)
118994	Diglycoaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9H-purin-9-yl)-	C^{14} (8)
139490	5-Methyltetrahydrohomofolic acid	C^{14} (methyl)
143647	DL-Alanosine	C^{14} (3)
148958	Ftorafur	C^{14} (2)
409962	1,3-Bis(2-chloroethyl)-1-nitrosourea	D (ethylene)

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table VII).

TABLE VII

CONTRACT LABORATORIES FOR PREPARATION OF RADIOLABELED MATERIALS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Monsanto Research Corporation	Yanko	N01-CM-23715
Stanford Research Institute	Tanabe/Leaffer	N01-CM-12162

Analytical-Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay, and purity. All lots of bulk materials to be used in toxicological and pharmacological or in clinical studies are sent for analysis before the materials are released for use or formulation. Also, quality control data is obtained for all formulated materials prior to their release to the clinical investigators.

It should be pointed out that the Cancer Treatment Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer compounds has become involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the presence of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research problem requiring the use of a combination of sophisticated techniques and the consideration of the stability of the compound and its possible contaminants or decomposition products.

In addition, the analytical unit is concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation, and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications."

The Unit collects and files such data as:

1. The detailed method of preparation;

2. The supplier's analytical sheet; and

3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the analytical-quality control contracts.

The assembled data is of use in writing purchase specifications. Attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1974, 196 analytical reports on 90 different compounds were received, representing 157 lots of bulk chemicals, and 68 lot of clinical formulations. Each lot is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicated unacceptable materials resulting in their being returned to the manufacturer for reprocessing.

Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compounds of interest to the Program.

The Section directed two analytical-quality control contract laboratories (Table VIII). In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE VIII

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Midwest Research Institute	Haggerty	N01-CM-33722
Stanford Research Institute	Lim	N01-CM-33723

Contractors Meeting

During the past year a Preparation Laboratory Contractors Meeting was held with representatives from all the Preparation Laboratory Contractors in attendance. The major objective of this meeting was for the different contractors in this area of endeavor to discuss mutual problems, techniques and procedures. In addition, the Section Staff had an opportunity to review administrative procedures and to inform the contractors of changes in the Program. Invited speakers made presentations of interest to the group and some of the contractors were requested to discuss topics of mutual interest. A favorable response to this meeting was expressed by all in attendance and additional meetings or workshops of the contractors in a specific area are planned for the future.

CHEMICAL AND DRUG RECORDS SECTION

DRUG DEVELOPMENT BRANCH

Scope

The responsibilities of the Chemical and Drug Records Section are (1) to acquire chemical compounds for the primary screen and for other program needs, (2) to develop, maintain and update systems of registry for all chemicals entering the program, (3) to maintain adequate storage and inventory of bulk chemicals and drugs, and (4) to coordinate the distribution of materials. These objectives are achieved through the efforts of staff members and the compound acquisition contractor to cultivate the interest of and to provide meaningful liaison with suppliers and potential suppliers; the surveillance of worldwide literature for new sources of chemicals and drugs; the distribution and utilization of chemical data; and the assurance that all discreet compounds obtained from commercial sources are maintained in accordance with the signed agreements.

Staff

During this report period the Section was staffed with two chemists, one biologist, and one secretary.

Acquisition of Compounds

Materials entering the screening program for the most part are voluntary contributions from research investigators at universities, government agencies, various non-profit institutions, industries and individuals interested in the area of chemotherapy. This continuous voluntary input of samples results from correspondence and personal visits of staff members to these various institutions, and the collection efforts of the compound acquisition contractor, Starks, C. P., Inc. This contractor, through diligent liaison efforts, was able to acquire approximately 35,000 samples of which approximately 85% were new to the program. The majority of compounds collected were from industry. The remaining samples were collected from industry. The remaining samples were collected from government and academic sources. A breakdown of the Starks C. P., Inc. activities show that 18 industrial sources, 15 academic sources and 3 government laboratories were contacted. An additional 23 potential suppliers have been contacted. As part of their acquisition efforts the contractor handled 1,351 supplier related transactions and correspondence. These transactions include letters of acknowledgment to suppliers, requests for refill samples, and requests for declassification of Discreet compounds. A concerted effort to acquire candidate screening samples more selectively has been initiated.

A total of 6430 new compounds were acquired as a result of efforts by NCI Liaison officers in Europe, Italy, Japan and England: 3881 compounds by the Institute of Jules Bordet, Belgium; 1620 by the Istituto Di Ricerche Farmacologiche, Italy; 251 by the Japanese Foundation for Cancer Research; and, 678 by Chester Beatty Research Institute in England. During calendar year 1974 the major portion of the liaison efforts were performed by the compound acquisition contractor and by NCI Liaison Officers in Europe and Japan.

During this reporting period 328 new suppliers were added, of which 264 were new non-industrial suppliers. Among the active compounds 50 new ring systems have been identified, bringing the total to 434 active ring systems.

The following chart gives a breakdown by sources for the new compounds registered in 1974:

COMPOUNDS SOURCES	NUMBER	PERCENT
Non Industrial	8286	20.8
Industrial	26254	65.9
Purchased	4121	10.4
Contracts	1153	2.9
	39814	100.0

To obtain 39814 compounds it was necessary to process approximately 48000 samples of which approximately 82% were non-duplicates.

Chemical Information

The Section cooperated with the Data Processing and Computer Systems Development Section in the registering of the total chemical file for computerized information retrieval. All new submissions are registered and duplicate checks are made automatically. Significant support has also been provided by Staff members in the development of the new chemical information system under contractual arrangements with the University of Pennsylvania. For rapid retrieval of chemical information, Master Chemistry Card Files and Molecular formula files are maintained. These files have been phased out with the advent of the automated system.

Chemical and Drug Distribution

This Section is responsible for the receiving, storage and distribution of bulk chemicals for screening. These activities are handled by Microbiological Associates, Inc. (Contract N01-CM-43793). Over 250,000 different materials are held in storage. These consist largely of chemicals and to a lesser extent plant products, antibiotics and steroids. A total of 22,937 new compounds were shipped to the screening laboratories; 8,658 refills and materials for special testing were weighed and 3,245 packages were shipped.

During 1974 approximately 31,454 samples were returned by the screening laboratories. These compounds for which testing has been completed, are entered into the inventory.

Other Related Duties

In compliance with the order issued by the Food and Drug Administration, the Section monitors and maintains records of input and distribution of materials considered by FDA as narcotics, barbiturates, hallucinogenic and other dangerous drugs. The FDA has specified that separate and accurate records be maintained for these materials and only those individuals with the proper narcotic license be approved to dispense and receive these items. To insure proper safety, the drug abuse compounds are secured in a separate file under lock and key.

The confidential status of compounds obtained from industrial sources requires constant updating and careful monitoring. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationshp but has made available many commercial samples not otherwise accessible. Frequently, there is a need to obtain the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agrees, in writing.

The Section has checked the publication status of the compounds reported in 150 manuscripts submitted for publication clearance.

Areas Under Development

Progress is being made to develop a coordinated approach in the selective acquisition of compounds. Programs under consideration include computerized searches of biologically active compounds with references and structures, analysis of chemical fragments of biologically active compounds, and feedback information from structure-activity correlation studies.

Plans for the installation of a new computerized inventory system is near completion and is expected to be operational by the summer of 1975.

Contracts

During the past year the Section has participated in the monitoring of the contracts listed in Table XIII.

TABLE XIII

CONTRACTS MONITORED BY THE SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Chester Beatty Research Institute	Connors	NO1-CM-43736
Microbiological Associates, Inc.	Berman	NO1-CM-43793
Starks C. P., Inc.	Foster	NO1-CM-53745

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the following major functions:

1. Development of investigational formulations for clinical use;
2. Production and packaging of investigational drugs;
3. Procurement by direct purchase of formulations for clinical use;
4. Quality control surveillance of all clinical dosage forms;
5. Preparation of Pharmaceutical Data Sheets for clinical brochures, IND's, etc.; and,
6. Storage and distribution of clinical dosage forms.

Staff

The staffing of this Section consists of three Public Health Service Pharmacists, one Ph.D. Visiting Fellow, two shipping and receiving clerks and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing chemical and physical data and a review of *in vivo* route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is provided with the route of administration, dose level, and available supporting chemical data. Consultation with the Project Officer is carried out concerning feasible formulation approaches.

Routinely, agents considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studied for the effects of pH, temperature, light and atmospheric oxygen. Additionally, compatibility tests are performed with buffers, preservatives and vial closures. Agents considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some parenteral dosage forms of potential antitumor agents. The contract with the University of Kansas is exploring these formulation difficulties. The contract is designed to perform research on current formulation problems and to design new approaches to parenteral drug delivery.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses.

The toxicological results and the accumulated pharmaceutical development data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1974, 23 projects for dosage form development were assigned. Ten of these involved new drug research and 13 assignments were for modification of existing preparations. A total of 13 new dosage forms entered clinical trial from the contract efforts, one of which was an original IND submission.

Production and Packaging of Investigational Drugs

An important function of the Section is to provide the Clinical Program with sufficient quantities of formulated materials. The production of dosage forms is mainly carried out by contract agreement with qualified pharmaceutical manufacturers.

The production output of clinical dosage forms for 1974 remained at a high level of effort. The formulation contractors produced a combined total of 79,195 bottles of tablets or capsules (1,185,570 oral doses) and 677,708 vials (parenteral doses). Production output which is dictated by clinical needs has continued to increase during the last few years. The production contractors have been able to meet the Program's needs for formulated materials.

Four production contracts and one development contract (University of Kansas) (Table XI) were monitored:

TABLE XI
CONTRACTS MONITORED BY THE CLINICAL DRUG
DISTRIBUTION SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ben Venue Laboratories, Inc.	Wickes	N01-CM-53811

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Iowa, University of	Wurster	NO1-CM-43743
Kansas, University of	Higuchi	NO1-CM-23217
Philips Roxane Laboratories	Marine	NO1-CM-12139
Tennessee, University of	Avis	NO1-CM-02084

Some contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. The Section encourages these contractors to develop and produce their own dosage forms. Specifications for formulation and production are provided to each contractor. This arrangement has proven economical, expedient and extremely beneficial to the program.

Dosage forms are developed and produced by pharmaceutical manufacturers in cooperation with the Division of Cancer Treatment. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between the Section and the manufacturer. During 1974, the following companies supplied a total of 46,726 injectables and 4,680 bottles of tablets and/or capsules through this joint research effort:

Bristol Laboratories, USA	NSC-26980	Mitomycin
Farmitalia, ITALY	NSC-123127	Adriamycin
Ives Laboratories, USA	NSC-82151	Daunomycin
Microbiological Research, ENGLAND	NSC-106977	Erwinia Asparaginase
Sandoz, USA	NSC-122819	VM-26
Sandoz, USA	NSC-141540	VP-16
Searle, USA	NSC-150399	Aldactone
Upjohn Company, USA	NSC-88536	Calusterone
Upjohn Company, USA	NSC-12165	Fluoxymesterone
Wellcome Foundation, ENGLAND	NSC-220537	C. Parvum
Winthrop Laboratories, USA	NSC-142982	Etrenol

Purchase of Dosage Forms

Another activity is the purchase of formulated products. These items fall into two categories: (1) investigational products and (2) commercial products. The investigational products are formulated proprietary drugs not available for commercial sale, but of interest to the Cancer Treatment Program. Such products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase. Procurement of all purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the size of the order and the priority for clinical need.

A total of 579,599 vials and 38,729 bottles of tablets and/or capsules (3,744,650 oral doses) of commercial and investigational dosage forms were purchased during calendar year 1974. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

Table XII summarizes the procurement activities of the Section during calendar year 1974.

TABLE XII
DRUG ITEMS PROCURED

SOURCE	Capsules/Tablets 1973	Capsules/Tablets 1974	Injectables 1973	Injectables 1974
Produced by Contractors	1,255,005	1,185,570	458,377	677,708
Contributed or Cost Sharing	208,710	600,800	37,679	46,726
Purchased Invest. Drugs	85,000	30,000	529,778	689,480
Purchased Commercial Drugs	2,035,300	3,744,650	247,946	579,599
	3,584,015	5,561,020	1,273,780	1,993,513

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, in collaboration with the Chemical and Drug Procurement Section, maintains a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator and sometimes by another laboratory.

Individual unit assays are required; the number of samples assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an outside analytical contractor to verify the analytical methodology and accuracy of the formulator. The confirmation analyses are mainly performed by the contract analytical laboratories, Stanford Research Institute and Midwest Research Institute.

This staff participates in evaluating the quality of chemicals and drugs. The analytical results of bulk chemicals and formulated clinical dosage forms are reviewed. During calendar year 1974, 401 lots of clinical dosage forms were reviewed.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under simulated clinical conditions to test the effects of temperature, light and salt additives.

The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf life surveillance program on all lots of non-commercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Three assay schedules are used for shelf life studies: Schedule A, designed to provide stability data under accelerated conditions; Schedule B, designed for controlled studies of repeat formulations; Schedule C is assigned to products that have been prepared more than three times. The Schedule C samples are held, but not assayed unless specifically requested. All Schedules provide a minimum two year surveillance record.

Preparation of Pharmaceutical Data Sheets

This Section prepares the Pharmaceutical Data Sheets for all investigational dosage forms used in the Program. The Data Sheets are used in preparing Clinical Brochures and for product information to pharmacists and investigators. The Sheets provide a description of the products and information on reconstitution (where applicable) and stability.

The Section also prepares Investigational New Drug (IND) attachments I, II, III, V and VII for submission to the Investigational Drug Branch for FDA-IND filing. These attachments consist of manufacture, control and packaging data required for investigational dosage forms.

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities are carried out by three shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year. During 1974, 1,282 foreign shipments were made. During the same period, 12,469 domestic drug shipments were made.

During 1974, a total of 2,072,708 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During that same period, 1,597,142 drug items were distributed (44% increase over 1973) averaging 49 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs, the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by a supply clerk and checked by the pharmacist prior to shipment. There are presently 13 such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on file by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgement to restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors.

During 1974, an average inventory of 115 dosage forms by lot number involving 201 different formulations were handled. These figures include investigational and commercial dosage forms.

Project No.: Z01 CM 03584-03
1. Division of Cancer Treatment, DR&D
2. Drug Development Branch
3. Clinical Drug Distribution Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Research in Dosage Form Development of New Antitumor Drugs

Previous Serial Number: 3584

Principal Investigator: J. Paul Davignon

Other Investigators: James C. Cradock, Aquilur Rahman, John S. Driscoll and Harry B. Wood, Jr.

Man Years

Total: 1.50
Professional: 1.50

General Objectives:

(1) Study new formulation approaches with potential utility for those compounds posing difficult formulation problems.

(2) Evaluate dosage form problems presented by clinical and laboratory investigators.

Specific Objectives:

(1) Formulation of an oral dosage form of NSC-71795 (Ellipticine).

(2) Development of parenteral dosage forms of NSC-79037 (CCNU) and NSC-95441 (Methyl CCNU).

(3) Solubilization of certain antitumor agents using non-ionic surfactant and non-aqueous solvent mixtures.

(4) Evaluation of pharmaceutical additives in the low temperature vacuum drying method.

Major Findings:

I. NSC-71795 (Ellipticine)

Hemolysis, hypotension and irritation at the injection site accompanied intravenously administered ellipticine in dogs and monkeys. Since ellipticine had consistently exhibited oral activity comparable to intraperitoneally administered drug in transplantable tumor systems, studies were directed toward an oral formulation. The solubility behavior of ellipticine with PVP over a molecular weight range of 10,000 to 360,000 was investigated as physical mixtures and in co-precipitated forms. Combinations of PVP of varying molecular weight can be used to affect rate of dissolution and solubility. If cardiovascular effects are related to peak plasma concentrations of ellipticine, these combinations may be suitable in providing a gradual release of ellipticine. Studies to elucidate the mechanism of ellipticine dissolution from experimental tablet formulations are being conducted.

II. Development of Parenteral Dosage Forms of NSC-79037 (CCNU) and NSC-95441 (Methyl CCNU)

Due to their poor aqueous solubility and stability, these nitrosourea compounds present a dual problem to the formulator of parenteral products. Surfactants increase the solubility of a variety of compounds by micelle formation and also protect certain agents from hydrolysis. Although stability precluded equilibrium solubility determinations, the apparent water solubility of CCNU and Methyl CCNU was increased 30 and 100 fold, respectively, in 5% polyethoxylated castor oil (PECO) and was adequate to deliver the anticipated dose by infusion.

The stability of both nitrosoureas in the solubilized system (5% PECO and 5% ethanol) was markedly increased versus water at various pH levels, surfactant concentrations and ionic strengths. Under simulated clinical handling conditions both nitrosoureas were stable (<5% decomposition) in commonly used I.V. fluids with pH <6.5 (5% Dextrose, Sodium Chloride and Lactated Ringers Injections). However, decomposition was rapid in 5% Sodium Bicarbonate. Both formulations meet criteria for activity and toxicity and have been prepared for clinical trial.

III. Solubilization of Certain Antitumor Agents Using Non-Ionic Surfactant and Non-Aqueous Solvent Mixtures

The applicability of solvent/surfactant mixtures in the solubilization of certain compounds exhibiting poor aqueous solubility was evaluated. The mixture usually consisted of equal parts of an organic solvent such as ethanol, dimethylacetamide or dimethylsulfoxide and the non-ionic surfactant, polyethoxylated castor oil. Experimental formulations of test compounds in the solvent mixture and providing clear solutions after 10 fold aqueous dilutions are considered acceptable candidates for further trial. Solubilized agents are re-evaluated in the tumor screen for activity and toxicity. This solubilization approach has been successfully employed with NSC-403169, acronycine; NSC-141549, acridinyl anisidide; NSC-146396, methoxynitidine; NSC-124921D; and, NSC-194200D.

IV. Evaluation of Pharmaceutical Additives in the Low Temperature Vacuum Drying Method

Low temperature vacuum drying ia a procedure with particular application to formulations requiring processing from organic solvents (usually ethanol). This procedure has been successfully utilized in the formulation of several parenteral antitumor agents such as BCNU (NSC-409962) and CCNU (NSC-79037). As in conventional lyophilization, an inert excipient is often required to provide mass in the vial, thus avoiding loss of the active component during the drying process. Urea and polyvinylpyrrolidone (PVP) were evaluated for use as excipients in the low temperature drying process. Evaluation of other pharmaceutical additives (buffers, chelating agents, antioxidants, etc.) is also ongoing.

Bruceantin (NSC-165563), an ethanol soluble plant product with potent activity (0.2-2 mg/Kg) was formulated with urea as the excipient. The experimental product is currently under evaluation.

V. Miscellaneous Projects

NSC-13875 (Hexamethylmelamine)

A gentisic acid complex of hexamethylmelamine has been developed under the University of Kansas contract that meets solubility and pH criteria. However, a lyophilized formulation suitable for large scale production could not be prepared due principally to sublimation of the active ingredient. Modifications of constitutents and lyophilization conditions were evaluated in the formulation laboratory. Inclusion of an excipient prevented sublimation and increased firmness of lyophilized cake without affecting solubility or stability.

NSC-146397 (Nitidine Chloride)

The solubility of nitidine chloride was studied as a function of additives (Tween 20, PVP, PECO), ionic strength, temperature and bulk lot. The solubility could be only marginally improved (two to three fold) by Tween 20, but was depressed by sodium chloride. Aqueous solubility was qualitatively proportional to purity of bulk lot. Minimal criteria (1 mg/ml) in 1% mannitol could be met with certain batches if heat were applied.

About eight other projects were carried out in the formulation laboratory in cooperation with other groups in the Division of Cancer Treatment. These included lyophilized and liquid fill preparations that were subsequently evaluated for pharmacologic and/or toxicologic effects.

Presentation:

"Use of Polyethoxylated Castor Oil in Formulations of Antitumor Agents for Intravenous Use", Parenteral Drug Association, National Meeting, October 31, 1974 (James C. Cradock).

KEYWORD DESCRIPTORS:
CCNU (NSC-79037)
Methyl CCNU (NSC-95441)
Emulphor
PECO
Polyethoxylated Castor Oil
Vacuum Drying
Non-Ionic Surfactants
Ellipticine (NSC-71795)
Formulation

DRUG DESIGN AND CHEMISTRY SECTION
DRUG DEVELOPMENT BRANCH

SCOPE

The Drug Design and Chemistry Section is primarily responsible for the intramural research of the Drug Development Branch. The objectives of this work are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, and (4) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis.

STAFF

The staff consisted of three scientists, one Staff Fellow, five NIH Visiting Fellows and a secretary. In addition, several persons are employed on the College Work-Study Program as part-time technicians.

SUMMARY OF ACCOMPLISHMENTS

Approximately five man-years effort was devoted to the intramural chemical research projects described in Individual Project Reports Z01 CM 03580, Z01 CM 03581 and Z01 CM 03582. An area of primary synthetic organic interest is central nervous system antitumor drugs (hydantoins, phenothiazines, quinones and procarbazine analogs). Analytical research centered on nuclear magnetic resonance studies. Structure-activity studies were completed for aziridinyl quinones as CNS antitumor agents, psychotropic drugs as CNS antitumor agents and rifamycin derivatives.

Significant accomplishments include the synthesis of a series of aminoaziridinylbenzoquinones which includes several members with confirmed activity (T/C > 200%) in the IP L1210 and P388 tumor systems. Confirmed activity also has been observed for these compounds in the intracerebral L1210, intracerebral P388, ependymoblastoma and IP B16 melanoma screens. The solubility problems associated with the earlier derivatives of this type appear to have been resolved with the preparation of these new compounds. Six hydantoin nitrogen mustards were synthesized for CNS antitumor testing. Several of these compounds were curative in the murine ependymoblastoma brain tumor system and reproducibly active in the IP L1210, P388 and B16 melanoma systems and the subcutaneous Lewis lung tumor system.

Section personnel were also involved with contract planning and served as project officers on five research contracts.

CONTRACT LABORATORY FOR STRUCTURE-ACTIVITY STUDIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Pomona College	Smith	N01-CM-33703

The contract with Pomona College has two objectives: (1) the application of regression analysis for the discovery of relationships between the structure-related parameters of potential anticancer agents and their antitumor activity and (2) an investigation of the molecular basis of drug action in enzyme inhibition systems. The Contractor is investigating the structure-activity relationships of analogs of (1) Adriamycin (2) thio-TEPA, (3) platinum complexes (4) 5-FU, and (5) acridine anisidides. In addition, the Contractor has measured electronic substituent constants for a number of Adriamycin derivatives. He has consulted with synthetic contractors and suggested analogs for synthesis based on structure-activity principles.

The investigation of structural factors affecting structure-activity relationships in enzyme-substrate systems has continued. Emphasis has been placed on a study of the dihydrofolate reductase system.

Section personnel served as project coordinators on a subcontract with the Entropy Limited Corporation under an Arthur D. Little continuing resources contract. The utility of the pattern recognition technique as applied to both congeners and structurally dissimilar antitumor agents is being evaluated.

Project No. Z01 CM 03580-06
1. DCT, DR&D
2. Drug Development Branch
3. Drug Design and Chemistry Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Chemical Research in the Development of New Anticancer Drugs

Previous Serial Number: NCI 3580

Principal Investigators: John S. Driscoll, John A. Beisler

Other Investigators: G. Peng, T. Krishnamurthy, F-T. Chou, K. Okada, M. Szekerke

Man Years: Total: 3.9
Professional: 3.9

Project Description:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. Organic synthesis research is directed towards the preparation of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the random screening program and the literature.

Specific Objectives:

1. The study of aziridinyl quinones, hydantoin, procarbazine, and phenothiazine analogs as potential central nervous system antitumor agents.
2. The investigation of the effect of protein complexation on antitumor drug activity, therapeutic index and drug stability.
3. The study of lung specific antitumor agents.

Major Findings:

A. CNS Target Directed Antitumor Agents

1. Aziridinyl Benzoquinones (Dr. Chou)

Solubility problems prevented exploitation of previously obtained active members of this family. Thirteen new compounds in the diaminodiaziridinylbenzoquinone family were synthesized during the past year. The amino series is new to the NCI program. Since optimum doses average 2.0 mg/kg, the aqueous solubilities, found to be in the 0.5-20 mg/ml range, appear more than adequate. The amino derivatives are also more active than any of the other aziridinylbenzoquinone families evaluated previously by the NCI. Among these amino compounds, L1210 and P388 activities of T/C > 200% are common. In addition, these derivatives had intracerebral L1210 activity as high as T/C 180% (confirmed). Cures in the ependymoblastoma brain tumor system were observed. The analogs were designed to allow a study of the redox potentials, steric effects and partition coefficients in a Hansch structure-activity correlation. Partially complete screening data is available on nine of the derivatives. All are L1210 active. Approximately four more compounds are planned in this series. Additional synthetic work will be carried out only if the structure-activity study on the completed screening data indicates further synthetic work would be profitable.

2. Hydantoin Mustards (Dr. Peng)

Additional samples of the three most active compounds in this series were prepared and biological testing was completed on the series. While only marginal L1210 activity was observed, confirmed T/C activities of 300% were observed in the IP P388 system and 275% in the IC ependymoblastoma screen. These compounds also possessed confirmed B16 melanoma (160%) and Lewis lung (150%) activity. No further work is contemplated in this series at the present time.

3. Bicyclic Hydantoins and Levamisole Analogs (Dr. Okada)

A new reaction was discovered during our study of CNS active hydantoins. This allowed the preparation of reactive hydantoins which appeared to be non-conventional alkylating agents. These compounds are oxygen isosters of Levamisole. In one approach, the 5,5-diphenyl groups of the parent hydantoin were replaced with less lipophilic alkyl groups. Several compounds (dimethyl, diethyl) were prepared and submitted for testing. Biological results are not yet available. If the first four target compounds are inactive, this line of research will be terminated. The relationship of these compounds to Levamisole may be investigated by a NCI group which has expressed interest in the possible immune response properties of the compounds.

4. Procarbazine Analogs and Hydrazine Derivatives (Drs. Peng, Krishnamurthy and Beisler)

A total of 16 aliphatic and aromatic procarbazine analogs and hydrazine derivatives were prepared and screened. None were L1210 active. Work is now being completed on two aliphatic analogs of procarbazine which should have approximately the same partition coefficients as the parent compound. This investigation will be completed with the synthesis and testing of these compounds.

5. Dibenzazepines (Dr. Peng)

Two dibenzazepine analogs, which were isosters of L1210 active phenothiazines, were prepared. Further work was discontinued when they proved to be L1210 inactive.

B. Antitumor Drug-Protein Complexes (Dr. Szekerke)

Dr. Szekerke's earlier work showed that proteins such as albumin and fibrinogen complex with antitumor alkylating agents to increase therapeutic index and drug stability. A project to determine whether similar results could be obtained with non-alkylating antitumor drugs was undertaken. Complexes (both covalent and charge-transfer) were prepared using albumin, γ-globulin, fibrinogen and chemically modified albumin as protein sources and 5-azacytidine, ara-C, dichloro-MTX, Actinomycin D, 4'-(9-acridinylamino)methansulfon-m-anisidide, Cytembena, ellipticine hydrochloride and AT-125 as antitumor agents. Thirty-five complexes were prepared, dialyzed, lyophilized, chemically analyzed and submitted for *in vivo* testing. Preliminary results on the first few samples indicate an increase in therapeutic index for some complexes.

C. Lung Specific Antitumor Agents (Dr. Beisler)

In response to a demonstrated need for drugs effective in the treatment of bronchogenic carcinoma a study was initiated to uncover potential leads which might provide direction in the synthesis of medicinals to improve the clinical prospects of humans with lung cancer. A three-fold approach to the problem was chosen:

The screening data summary file of the Drug Evaluation Branch was searched for all those compounds submitted to the program which had confirmed activity (T/C > 125%) in the Lewis lung carcinoma test system. Approximately 60 compounds which had been entered into the program met these requirements. Structural leads are being sought among these compounds.

A list of drugs which have shown promising activity in the treatment of any lung disease was prepared. About 25 drugs with clinical activity against pulmonary tuberculosis already had been screened in the DR&D program. Few of these have been tested in the Lewis lung assay. These tests will be scheduled.

An effort is being made to compile a list of compounds which have an affinity for and concentrate in lung tissue, giving rise to either a therapeutic or toxic effect. Letters requesting samples of a number of drugs of this type have been sent to several pharmaceutical companies.

KEYWORD DESCRIPTORS

Synthesis, antitumor, quinone, hydantoin, procarbazine, CNS, protein-drug complex, lung.

PUBLICATIONS

Khan, A.H. and Driscoll, J.S.: Active Antitumor Components in a Decomposed Amino Sugar I: Effect of Sugar Structure on Activity. J. Pharmaceutical Sci. 64: 295-299, 1975.

Peng, G., Marquez, V.E. and Driscoll, J.S.: Potential CNS Antitumor Agents. Hydantoin Derivatives. J. Medicinal Chem., in press.

Project No. Z01 CM 03581-06
1. DCT, DR&D
2. Drug Development Branch
3. Drug Design and Chemistry Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Analytical Chemistry of New Anticancer Drugs

Previous Serial Number: NCI 3581

Principal Investigator: John S. Driscoll

Other Investigators: Li-Ming Twanmoh, J. A. Beisler, D. Cooney

Man Years: Total: 0.1
Professional: 0.1

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;

2. establish the identity and purity of bulk and formulated drugs;

3. determine important physical and chemical properties of new anticancer drugs; and

4. study reaction mechanisms and structure-activity relationships.

Major Findings:

A. Enzyme-Drug Binding Studies [Drs. Twanmoh, Cooney (NCI-LT), Cohen (NIAMD)]

A nmr study of the structural factors influencing the binding of aspartic acid impurities to L-asparaginase was completed. Deuterium exchanged derivatives of aspartic acid were employed.

B. Inhibitors of Asparagine Synthetase [Drs. Beisler and Cooney (NCI-LT)]

The observation that the crude mixture from the diazotization of L-asparagine was inhibitory to the enzyme asparagine synthetase prompted an analytical study of the mixture. Upon repetition of the reaction, a component identified as 2-hydroxysuccinamic acid was isolated in 77% yield. Neither this compound nor this crude reaction mixture, however, was enzyme inhibitory. Experiments to obtain an active mixture are in progress.

KEYWORD DESCRIPTORS

Enzyme complex, L-Asparaginase, Asparagine synthetase.

Project No. Z01 CM 03582-01
1. DCT, DR&D
2. Drug Development Branch
3. Drug Design and Chemistry Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Structure-Activity Studies of Anticancer Drugs

Previous Serial Number: None

Principal Investigators: Frank R. Quinn, John S. Driscoll

Other Investigators: R. Geran, N. Melnick, C. Maloney

Man Years: Total: 1.2
Professional: 1.2

Project Description:

General Objectives:

The objectives of this project are:

1. the investigation of structure-activity techniques as applied to antitumor data;

2. quantitative correlations of screening data with structural features among antitumor agent congeners;

3. quantitative and qualitative correlations between structure and antitumor activity among large numbers of structurally unrelated compounes;

4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Project Description:

A. Quantitative Structure-Activity Relationships (QSAR) by the Hansch Technique (Dr. Quinn)

   1. Intramural Research

      a. Sixty-three families of compounds were identified as having potential for quantitative structure-antitumor activity studies. This was accomplished by reviewing the structures of all the compounds in the DR&D program with confirmed activity.

b. Terephthalanilides

Biological and structural data were acquired for over 100 active members of the series and dose response curves were generated for 18 congeners. These data were analyzed with the aid of the Hansch program. The initial correlation equation indicates increased activity is related to decreased lipophilic character. An attempt will be made to correlate the toxicities of these materials to see if toxicity can be minimized while maintaining activity by means of the proper structural manipulations.

c. Quinazolinones

A quantitative correlation relating activity with increased lipophilicity was obtained for 14 compounds in the L1210 cell culture system. Since the major problem with this series of compounds is poor solubility, more lipophilic derivatives would be of little value and this study was terminated.

d. Ellipticine Derivatives

A good correlation was obtained in two variables for the L1210 (Q4D) *in vivo* system. Unfortunately this regimen, which had the most test data available, included only seven compounds. Additional compounds must be tested before statistical significance can be placed on a two variable equation. A cooperative effort with Stanford Research Institute is under study.

e. Cyclophosphamide Analogs

Both structural and biological data were obtained from the files for this series. Not enough *in vivo* data on any one regimen was available to attempt a quantitative correlation.

f. Miracil D Analogs

Structural and biological data have been obtained for this series. Correlation work is in progress.

2. New QSAR Data Handling Techniques

In cooperation with the Drug Evaluation Branch, DR&D, and DCRT, existing computer programs were modified to provide screening data in special formats for efficient use in QSAR studies. A plotting program which graphs T/C vs dose has also been made operational. A Free-Wilson computer program was installed and is available for use on appropriate classes of compounds.

3. Cooperative Studies

Using the data handling techniques described above, biological data were obtained from the biological file and pre-analyzed along with structural information. Families of compounds with sufficient data to be sent to Dr. Hansch (Pomona College) for analysis under contract were adriamycin, TEPA, 5-FU and the platinum derivatives. The first three are under study. The structural data from the platinum derivatives were found to be inappropriate for a Hansch type study.

B. Other Structure-Activity Studies

1. Psychotropic Drug Study (Ms. Melnick, Lomax, Mr. Ing, Dr. Quinn)

The initial antitumor screening was completed on the 320 CNS agents obtained from over 60 pharmaceutical companies. Forty-two compounds were active in at least one test in the L1210, P388 or B16 models. Leads in the β-phenethylamine, phenothiazine and butyrophenone families were generated.

2. Quinone CNS Study (Dr. Geran)

Eighteen aziridinyl quinones were studied in depth for activity in IC and IP L1210 and P388 systems, as well as the IP B16 and IC ependymoblastoma tumor models. Several compounds show excellent activity in a majority of the tumor systems.

KEYWORD DESCRIPTORS

Structure-activity, Hansch, antitumor, terephthalanilides, quinazolinones, ellipticine, cyclophosphamide, Miracil D, adriamycin, TEPA, 5-FU, platinum, psychotropic, quinone.

PUBLICATIONS

Driscoll, J.S., Hazard, G.M., Wood, H.B. and Goldin, A.: Structure-Antitumor Activity Relationships Among Quinone Derivatives. Cancer Chemother. Reports, Part 2, No. 2 4:1-362, 1974.

Quinn, F.R.: Structure-Activity Techniques in the Treatment of Antitumor Drug Data Symposium. Forward. Cancer Chemother. Reports, Part 2, No. 4 4: 31, 1974.

Driscoll, J.S.: Structure-Activity Techniques in the Treatment of Antitumor Drug Data Symposium. Introduction and Overview. Cancer Chemother. Reports, Part 2, No. 4 4:33, 1974

Driscoll, J.S.: Quinones as Anticancer Agents Symposium. Quinone Structure-Activity Relationships. Cancer Chemother. Reports, Part 2, No. 4 4:3-4,1974.

Quinn, F.R., Driscoll, J.S. and Hansch, C.: Structure-Activity Correlations Among Rifamycin B Amides and Hydrazides, J. Medicinal Chem., in press.

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Section is primarily concerned with: (1) the implementation of a new, automated Chemical Information System (CIS) which supports such activities as the accessioning, storage, and distribution of compounds acquired for antitumor testing; (2) operating the CIS; and (3) coordinating many of the activities related to the CIS. In addition, the Section cooperates in projects to explore the usefulness of automated literature and chemical files maintained by federal agencies, industrial organizations, and information vendors.

Staff

The staffing of the Section consists of two system analysts and one secretary.

Emphasis of Effort

During 1974, the Section concentrated on monitoring the: (1) operation of the first phase of the new Chemical Information System; (2) implementation of the second phase of CIS; and (3) conversion of the manual, inventory control records to an automated file. In these efforts, the interaction with the system by the Drug Development and Drug Evaluation Branches and the contractors engaged in such activities as system implementation and operation, acquisition, and storage and distribution was coordinated.

Operation of the Phase I Chemical Information System

The first phase of the system became operational at the Chemical Abstracts Service (CAS, the operation contractor) in July, 1974. This phase of the system handles a wide range of chemical and drug sample processing activities, including the input of chemical structures with other data, identification of duplicates, assignment of accession numbers, and providing various required reports. The System has accelerated the accessioning process and has provided automated operational and management control over the interrelated contractual activities.

During the first year of the system operations contract, approximately 34,000 acquisitions had been registered. As of the end of 1974, the registry file had 265,000 unique chemical structure records and the nomenclature file had 468,000 chemical names, including CAS Registry Numbers.

The information retrieval component of the system became operational in Fall, 1974. Using terminals with simplified, computer-supported techniques, the system permits access to the files for full structure search (to secure an immediate answer for a possible duplicate sample) and substructure search (for analogs), including the retrieval of associated, non-structural information. The new system provides more rapid and current duplicate checking, outputs structures, and using teleprocess techniques, retrieves information from the chemical file virtually immediately as requested.

Implementation and Installation of the Phase II Chemical Information System

The University of Pennsylvania is scheduled to implement and install the second phase of the CIS at CAS in July, 1975. This phase will extend the system by providing for automating shipping procedures, inventory control, and the transfer of machine-processable information related to accessions, shipments, and other activities to the automated Biology System. Submissions that are duplicates or are closely related to known active compounds will be automatically identified for non-routine testing.

Miscellaneous

The Section coordinated the reporting of the Drug Research and Development planned requirements for the NIH Automated Data Processing and Management Information Reporting System.

In the coming year, the effort will be concentrated on administering and managing the contracts with Chemical Abstracts Service and the University of Pennsylvania. The CAS contract will include the operation of the Phase II of the System and the assumption of system maintenance (programming) responsibility. The University of Pennsylvania contract will provide for completing the system documentation, maintaining the system pending the training of CAS staff to assume this responsibility, and for improving retrieval efficiency.

TABLE XIV

CONTRACTS MONITERED BY THE SECTION

CONTRACTOR	PRINCIPAL INVESTIGATOR	CONTRACT NO.
CHEMICAL ABSTRACTS SERVICE	Seals	NO1-CM-43722
UNIVERSITY OF PENNSYLVANIA	Lefkovitz	NO1-CM-33719

NATURAL PRODUCTS SECTION
DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from higher plants, animals, and microbial sources. This area will be discussed under the headings: Plant and Animal Products; and Fermentation Products.

Staff

The Section has operated with a staff of four persons, consisting of two professional (Ph.D level) and two clerical employees. Dr. Hartwell, the Natural Products Head, retired in March. Dr. John Douros was appointed Acting Head of the Section.

Surveillance

World-wide surveillance of the published literature and collection of chemicals and drugs for biological evaluation are carried out in the Natural Products area. An informal literature survey has been conducted which has yielded several interesting compounds to the program.

Accomplishments

The Natural Products Section continues to contribute an increasing number of compounds to the program, many of them of novel structure. Those with the highest activity will be submitted for confirmation and testing in the slow-growing tumors, B16 melanoma and Lewis Lung carcinoma.

Table II lists the materials of natural origin acquired during the year under review compared with the previous year. Of the crude extracts, contractors provided 95% of the 725 fermentation products, 65% of the 2,011 animal products and 85% of the 5,830 plant products. A total of 274 purified (mostly crystalline) natural products were received or isolated. Seventy-three were active and twenty-five passed the criteria for confirmed activity (Step III). Four fermentation compounds were assigned to Decision Point IIA. Two fermentation compounds were assigned IND numbers: Corynebacterium Parvum Vaccine (NSC-220537) and Mycobacterium Tuberculosis Vaccine BCG (NSC-B116327).

Steps have been taken to make more selective the plant collection program resulting in a greater yield of active plants from a smaller total number, thus reducing the screening requirements. The experiment initiated two years ago to improve the plant extraction procedure and give higher yields of actives was successful. The new extraction procedure has now been adopted. The fermentation program showed a marked decrease in primary screening but a marked increase in actives (24%). This is due to extensive use of in vitro screening tests before submitting beers for animal testing. All these improvements are outlined in more detail in the appropriate following paragraphs.

PLANT AND ANIMAL PRODUCTS PROGRAM

In carrying out its objectives in the plant and animal products area the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (a) plant and animal collecting; (b) preparation of extracts; (c) fractionation of active extracts, and the isolation and identifications of the active agents therefrom; and (d) in collaboration with the Chemical and Drug Procurement Section, the preparation of large amounts of active agents from plant materials for preclinical toxicology and clinical trial.

Through extensive correspondence, contact is maintained with potential domestic and foreign suppliers in order to acquire plant, animal and fermentation compounds which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

The following contractors are engaged in the Plant and Animal Products Program (Table IX).

TABLE IX

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Agriculture, U.S. Department of	Perdue	Y01-CM-40001 (transfer)
Arizona, University of	Cole	N01-CM-33750
Arizona State University	Pettit	N01-CM-12308
Central Drug Res. Inst.*	Anand	01-004-1 (PL-480)
Hawaii, University of	Sagawa	N01-CM-33747
Illinois, University of	Farnsworth	N01-CM-22078
Research Triangle Inst.	Wall	N01-CM-92019
Virginia, University of	Kupchan	N01-CM-12099
WARF Institute, Inc.	Coon	N01-CM-43707

*Joint with Synthesis Program.

In addition, an open-end, fixed-priced contract for the procurement of plant extracts has been continued with the University of Costa Rica (Dr. J. Saenz R.) and a similar one for the procurement of marine animal extracts has been continued with the University of Oklahoma (Dr. A. J. Weinheimer). Beside the contractors listed above, several groups, including grantees and those not supported by the NIH, contribute in different ways to this program.

Progress was made in the following areas of plant, animal and fermentation products:

A. Plants

1. Nomenclature Review. Good progess has been made by the U.S.D.A. in reviewing the 79,000-odd botanical names in our plant accessions to assure technically correct Latin names. This will (1) eliminate synonymy, thereby avoiding future conflicts where the same plant under two different names is being fractionated, (2) bring the nomenclature in line with other large collections, including that of the U.S.D.A., thus making computer analyses of data more significant, and (3) provide accurate names for the future publications of the fractionators and others using the file. The U.S.D.A. is coordinating with NCI's Automated Information Section to rectify any variations in plant nomenclature.

2. Program Monitoring. This is a continuing joint project of the U.S.D.A. and the Section to review the total plant input and the chemical output of the program for the purpose of recognizing plant families that (a) are known to produce compounds of interest to our program or that have been inadequately screened, in either case making them deserving of special effort in collecting, and (b) are known not to produce compounds of interest to our program, or that have been adequately screened, in either case making them subject to avoidance in collecting.

This selective collection has been inplemented for some time and is yielding promising results. Plants which belong to families which have produced compounds of interest are being collected in large quantities suitable for extraction without the necessity for recollection, and already the indications are that a substantially higher yield of confirmed actives is being obtained. A planned program to collect in countries known phytogeographically to be rich in such families is being pursued.

Thus, by this means, a rational approach to plant collecting has been developed, combining guidance provided by chemical feedback with modified exploratory collecting necessary to locate new areas. Such an approach was not possible earlier until our data bank had become more extensive. Our future collecting will be substantially more responsive to the program needs.

3. Duplicate Searching. Following the establishment of a computer "terminal" at the U.S.D.A., it is possible for the U.S.D.A. to communicate telephonically with our plant genus and species "bank" and instantly determine duplicates among accessions. Duplicates would be reviewed to determine if they should be evaluated. This procedure could make a great saving in the expense of extracting and screening these plants.

4. Large Re-collections. One large plant re-collection was made for the preparation of clinical quantities of drugs or of relatively large amounts of drugs for advanced testing. The U.S.D.A. collected *Bouvardia ternifolia*, whole plant (for compound B-613763 F049), 10,000 lbs. from Mexico for NCI.

5. Improvements in the Plant Extraction Process. The "500 plant experiment" to identify a better extraction process for obtaining active materials from plants was successful. Although all the data are not in, the results obtained show we obtain 12 times more actives using the new extraction procedure and greater than 50% of these have T/C greater than 150 vs PS. This indicates that we should evaluate the genus and species of those plant which were inactive under the old extraction procedure as we may have missed many potential plant materials with antineoplastic activity.

6. Plant Statistics. One hundred and sixty-seven genera and four hundred and ten species were fractionated this year. Five hundred and forty-nine extracts were found active in PS or KB. Three hundred and fifty-nine PS and one hundred and eighty-seven KB extracts were found to have confirmed activity. One hundred and four crystalline plant products were obtained of which eighty-six were from contracts.

B. Marine Organisms

During the year, 2,011 animal products, mostly marine, were accessioned. Seventy-six crystalline materials were obtained from this work and are in various stages of testing. Eleven of these were from our contract and sixty-five were obtained by surveillance. About 200 different species active in Leukemia P388 or KB cell culture, and about 34 species are currently under fractionation. This has proved to be an unusually difficult field chemically, due probably to the nature of the active agents and the accompanying impurities. We are presently reviewing our outstanding leads in this area and will attempt large collections of these materials to allow the obtaining of enough active material for evaluation in our four principal tumor systems.

FERMENTATION PRODUCTS PROGRAM

Antibiotics have a great potential in cancer chemotherapy as primary treatment agents, in combination or as the initial structural building blocks for new and novel compounds that can be synthesized and used in the clinic.

The present fermentation program has been carried out through contracts at different biological laboratories listed in Table X:

TABLE X

FERMENTATION CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Claridge	N01-CM-43759
Georgetown University	Katz	N01-CM-43732
Litton Bionetics (Project 17)	Langlykke	N01-CM-23294*
Michigan Dept. of Health	Olson	N01-CM-33702
Parke-Davis & Co.	Dion	N01-CM-43754
Upjohn Co.	Neil	N01-CM-43753

*One of 20 plus projects under the Litton contract.

A computer analysis of the activity in PS of all the microbial cultures tested in our program revealed some extremely active fermentations.

Fifty-five culture fermentations are under chemical fractionation by either the fermentation contractors or the contract plant fractionators, and it is expected that some will be purified and evaluated in the coming year. Several of the high priority culture fermentations have been assigned to plant fractionators for isolation of the active agents in order to reduce a slight backlog with the fermentation contractors and to take advantage of the expertise of the plant fractionators. Already three pure compounds have been isolated by our plant fractionators from antibiotic fermentations. Additional antibiotics will continually be obtained from our surveillance program and will be evaluated.

Bristol Laboratories. This fermentation contract is to evaluate the potential of biotransformation and co-oxidation. The substrates to be used are different from those being evaluated by several other contractors. Twelve successful biotransformations have been identified and isolation of these compounds is underway.

Georgetown University. This contract is designed to obtain novel actinomycins using biotransformation and co-oxidation techniques. Five new actinomycins have been isolated and are being evaluated in NCI in vivo tumor tests.

Project 17. NCI has initiated a fermentation project at the Frederick Cancer Research Center, Frederick, Md., to take advantage of existing fermentation and analytical facilities to investigate the full potential of fermentation in the cancer chemotherapy field.

The pilot plant program is to be used to produce larger quantities of antineoplastic antibiotics which have been obtained from our present contracts or in house research at FCRC. The pilot plant will obtain engineering, microbiological and chemical data on the various processes and do the necessary developmental research to improve and decrease costs of the antineoplastic agent. Limited pilot plant capacity has been a major drawback in our present contract fermentation program. Materials needed for chemical purification, advanced testing, etc. have not been more rapidly forthcoming because of direct competition for facilities with the pharmaceutical companies' own research program. The building of this facility is scheduled for August, 1975.

The fermentation research program is to explore new or expand certain areas which are not being thoroughly evaluated and are as follows:

a. Microbial biotransformation of antibiotics, plant materials and synthetic chemicals which have demonstrated limited activity, toxicity, solubility problems, etc. will be evaluated. It is anticipated that these problems may be overcome by the use of this technique or completely new materials may be produced.

b. Evaluation of thermophilic, psychrophilic, osmophilic and halophilic, organisms as potential producers of antineoplastic agents.

c. Utilization of unusual substrates to obtain unique organisms and evaluate their fermentations.

d. Co-metabolism of (a) and (c).

e. Continuous fermentation of known producers of antineoplastic antibiotics, to reduce the costs of the process and to select better strains of organisms.

f. Fixed culture fermentation of various antineoplastic-producing organisms to produce novel materials.

g. Mutation of strain selection, stimulants and enzyme inhibitors will be evaluated to see if the metabolism of organisms is radically changed in order to produce more or different antibiotics.

h. Development of several enzyme products which are of interest to the program.

Michigan Dept. of Health. This is a tissue culture laboratory contract which is used to assay fermentation broths, chemical fractions, and media studies for their antineoplastic activity. The Michigan Dept. of Health has an active fermentation program that yielded eight crystalline antibiotics this year which are being tested for their anti-cancer activity.

Parke Davis & Co. This contract is designed primarily to find novel antineoplastic antibiotics by using unique cultures and novel fermentation techniques. In addition, this group has developed a Daunomycin process for NCI. One crystalline antibiotic was obtained and is presently being evaluated.

Upjohn Company. This contract includes fermentation, in vitro and in vivo assaying, chemical isolation, pharmokinetics and biochemistry. Biotransformations are being conducted on different antibiotics. The technique primariy being used is anaerobic and the substrates are different from any of our other contracts. Two novel crystalline antibiotics were found to be active this year and extensive evaluation is underway.

Future Plans

Three groups of organisms have been shown to be the principal producers of antibiotics; they are bacteria, fungi, and actinomycetes. There are still multitudes of new and different organisms to be isolated using unusual substrates, radical environmental conditions and various unique soils and isolation techniques. In addition, various fermentation and genetic techniques should stimulate the production of more and unique antineoplastic antibiotics. Two of these techniques have been introduced into our contract fermentation program; co-metabolism and biotransformation. Their possibilities are limited only by the ingenuity of the microbiologists. Already more than 200 organisms which can utilize unusual substrates have been isolated.

There are several areas of fermentation that have not been explored for their ability to produce new antineoplastic materials. Among these are (a) the use of a variety of different carbon sources for fermentation substrates and as co-oxidizing materials which we are now pursuing; and (b) the study of yeasts, of thermophilic and psychrophilic microbes, and of mixed cultures which will be evaluated in the near future.

SCIENTIFIC RECORDS SECTION
DRUG DEVELOPMENT BRANCH

Scope

The Scientific Records Section (SRS) is responsible for (1) managing the storage and retrieval of chemical information within DDB; (2) investigating and implementing new techniques and products to make this information more useful and (3) preparing shipping documents for all new materials and coordinating the shipping of compounds of special interest.

Section personnel update and monitor the quality of the master structure file, the supplier name and address file and the non-structural data files (i. e. names), and have the capability to search these files by structure, structural fragment, name or NSC number. Retrospective searches of this file are used in structure activity studies within DR&D and in decisions on the adequacy of development of a given class. Cooperation and consultation are given by members of the Section in changes involving the chemical and biological information systems since the Section is both participator and user in these systems, and outside information resources are monitored for developments that could be of interest to DR&D users. Once checked for novelty, all compounds new to the screen pass through SRS where instructions for routine or special testing and where to screen is coordinated from the appropriate individuals in DEB and DDB.

Staff

The staff of the Scientific Records Section consists of 3 chemists, one chemical information clerk and 3 clerk typists.

Shipment of Compounds to Screeners

Material Shipping Lists (MSL) are prepared for new compounds prior to their shipment to the screening laboratory. The MSL provides the initial input to the biological data processing system. Shipping lists for 26,481 compounds were prepared during calendar year 1974, and the testing instructions of special compounds were coordinated. Assistance was provided in the development of the new shipping subsystem to be implemented in 1975 as part of the computerized Chemical Information System. All duplicate structures are cumulated and sent to DEB for review of testing, adequacy of testing and further testing.

Supplier Records

The manual record keeping system for 4,000 suppliers' names and addresses was converted to a computer records system during the past year. Cards containing the names and addresses of all individuals or companies who have ever supplied drugs to DR&D were printed by computer and reviewed and filed, and this master file is now corrected and updated by SRS personnel. A separate computer name and address file used primarily in the biological file mailing was continued until automatic links to the new system were established.

Name and Formula Indexes

The name and formula indexes to the chemistry file produced under contract at Chemical Abstracts Service (CAS) are checked and set up for use by SRS. Synonym services such as Unlisted Drugs and the U. S. Adopted Names Service are made available to DR&D users. The Section also subscribes to Cancerline and Chemline, two on-line literature and name retrieval services at the National Library of Medicine, in order to additionally augment its ability to translate synonyms into structures to determine presence in the NCI file. The 32,000 page name and formula indexes (370,000 names) were produced on 16mm microfilm using computer output microfilm instead of paper for the first time, thus allowing compact storage and easier access.

Master Chemistry File

The master files were filmed on a continuing basis throughout the year and in addition the discontinued manual molecular formula file was filmed and the originals discarded. The Section began coordinating the filming of the DDB contract progress reports and certain formulation data in order to more efficiently utilize these files. In addition to the hand-drawn chemistry card used as the master record for several years, a 5 x 8 card is now produced completely by computer in the new chemical system with structure, supplier and handling information, and systematic name. As a new record type, 9,500 of these were reviewed by a chemist to check the quality of output before filing. Section personnel also participated in the total review of all systems outputs for the startup of the new chemical system, as well as quality control for the routine outputs. Changes in programs and procedure were made to the CIS based on these large reviews.

Substructure Search

The new interactive substructure search system, developed under contract at the University of Pennsylvania, was installed at the Chemical Abstracts Service as the replacement for the former batch processing system. The installation of terminals, printers and communication equipment at DDB, and the actual running of tests were carried out by SRS personnel beginning in December 1974. After cooperative resolution of problems, actual questions were switched to the new system. The ability to code interactively and run immediately afterwards has resulted in faster response time to queries, with more comprehensive results. Seventy substructure searches of the full file of 250,000 structures were made. As an example, a comprehensive survey of the active/inactive totals of all metals in the program was developed for the Drug Evaluation Branch. Seventy-five searches for active analogs were run primarily for the purpose of aiding the Drug Evaluation Committee and Decision Network meetings in evaluating the biological data of a compound in light of its structural novelty. Answers to 35 queries for listings of compounds by a given supplier and approximately six hundred retrievals of individual compounds from structures or names were provided. Results of these queries were given to requesters as copies of the chemistry card, for the most part, in order to provide structural output.

Special Projects

Investigation of techniques to provide more selective acquisition of agents were continued throughout the year. They included:

(a) Continuation of research into the use of statistical techniques with chemical structural fragments. Section members cooperated with the Drug Design and Chemistry Section in a small contract with Arthur D. Little to investigate clinically active compounds and nucleosides using hand-coded chemical fragments. In addition data and advice was provided to Dr. Louis Hodes, DR&D, in investigating the utility of the fragments generated for the new CIS. The class of methods being investigated give some promise for success in being able to model the probability of a compound being active; however, more basic work in selecting fragments remains to be done.

(b) Coordination of a literature survey for biologically site-directed agents. A preliminary literature search for chemicals localizing in specific tissues was made with the rationale that agents with activity in specific tumors might be found. The initial results were too broad and work is now going on to narrow the search criteria.

ANNUAL REPORT

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Drug Evaluation Branch mission is to develop and coordinate laboratory projects for the recognition, recommendation, and preclinical development of improved anticancer therapy. This is achieved by (a) screening large numbers of synthetic and natural products for biological activities indicative of clinical usefulness; (b) evaluating "active" drugs for conditions of use that may enhance their efficacy - e.g., modifications of treatment schedule, combination chemotherapy, and combined modality treatment; and (c) developing improved methods for preclinical drug evaluation and for solution of problems arising during preclinical toxicological studies and clinical trials. Drugs selected for development are studied in-depth with regard to spectrum of antitumor activity - e.g., against "fast" and "slow" growing tumors; laboratory models of specific human tumors; drug resistant tumor variants, toxicity in rodents; biological activity of clinical formulations, activity against specific phases of the cell life cycle, and specific biochemical sites of action. DEB collaborates closely with the Drug Development Branch, DR&D, to provide information pertinent to the synthesis of new drugs and with Experimental Therapeutics, DCT, to study drug metabolism and physiological disposition, and the role of drug interactions on the effectiveness of combination chemotherapy. Program is implemented through contracts with highly qualified institutions, projects conducted in DEB's intramural laboratories, and through collaborations within and outside of DCT. Results provide information essential to fulfillment of tasks set forth in the DCT array of program activities. Studies are directed to achieving the objectives of NCI and the National Cancer Plan and to elucidating scientific principles that have a major impact on approaches to these objectives.

DEB includes the Office of the Chief (J.M. Venditti), and four Sections: Automated Information (J.F. Waters, Head); Biochemistry (F.R. White, Head); Molecular Biology and Methods Development, MBMD (V.H. Bono, Jr., Head); and, Screening (B.J. Abbott, Head). It is staffed by 15 senior professionals including three commissioned officers, one staff fellow, and two visiting fellows, and 17 junior professional, technical, and clerical personnel. Five of the latter are part-time or employed under STRIDE.

During the past year DEB was responsible for the direct management of 37 contracts and for monitoring drug evaluation tasks performed under ten contracts managed by other DCT units (Table 1). An analysis of each contract relating individual tasks to funding and a brief description of the work scope can be found in the current report of the Director, DCT. Table 2 of this report summarizes the distribution of DEB monitored contract funds by project area and DCT-array. DEB intramural laboratory projects are conducted within the MBMD Section and have emphasized the development of techniques for the analysis of drug effects on the progress of proliferating cells through their life cycle (current Project Report No. Z01-CM-03579-02-DEB) and for modification of cell surfaces as a means of limiting cell division and increasing immunogenicity (current Project Report No. Z01-CM-03583-04-DEB).

In calendar year 1974, 48,380 new materials were submitted for primary screening, and 355,377 screening tests were conducted in tumor bearing rodents; a test being defined as one group of three to ten drug-treated animals. There were 9,215 tests for toxicity in normal animals and 35,871 in vitro tests. Of the in vivo antitumor tests, 60 percent were against mouse leukemia L1210 used for initial screening of synthetic agents, 35 percent against mouse leukemia P388 used for initial screening of crude natural products and for bioassay of natural product fractions, and 5 percent in secondary screens including melanoma B16, Lewis lung carcinoma (LL) AK lymphoma in mice, Walker 256 carcinoma in rats, and miscellaneous tumors. These figures exclude those tests related to the detailed evaluation of drugs, drug combinations, and combined modality treatment which are not yet computer processable because of the specialized nature of the experimental design. Nine hundred nineteen compounds met minimal criteria for "activity" in primary or secondary screens, 218 in in vivo systems. Seven hundred fifty-five were reviewed for conformance to more stringent criteria required to be recommended as potential clinical candidates. Seven passed Decision Point (DN) 2A of DCT's linear array and proceeded to clinical development (see Table III of the current report, Screening Section, DEB).

The selection of models for primary and secondary screening is a major task of DEB. Ideally, one might wish to test all new compounds in a wide spectrum of models including those of established predictive value for the clinic as well as new, as yet unevaluated, rationally selected systems. Past attempts to approach this ideal were hindered by the small amounts of many of the compounds submitted to the screen and by a limited screening capacity. The problem has been intensified in recent years by an increase in both total submissions and the proportion of the total received in small amounts, as well as by attempts to maintain screening capacity at reasonable levels in the face of cost inflation; factors which preclude broad spectrum primary screening in vivo. Consequently, it has been necessary to select one model for initial screening, despite our recognition that no single existing model can predict perfectly therapeutic efficacy in man. Wider spectrum or secondary screening has been reserved for relatively few materials that are "active" in our primary screen or other screening programs, and for compounds of special interest based on the rationale for their synthesis. Primary screens have been selected because of their sensitivity to agents of known clinical value, and secondary screens because they exhibit some biological characteristic different from those of primary screens, e.g., tumor cell growth kinetics or histopathological resemblence to specific human tumors. Since secondary screening requires testing for 5 percent or less of total compounds submitted, a wider spectrum of assays can be used and the required amount of each material can usually be acquired. An obvious disadvantage to this approach is the requirement that randomly acquired materials must "pass" a limited primary screen to be a candidate for wider spectrum evaluation. Thus, if the input of new materials is to remain high and essentially unrestrained with regard to compound class, if there is no restriction on the minimum amount of a material that will be accepted, and if we continue to use an in vivo model for primary screening, the selection of that model is critical.

Approximately 250,000 synthetic materials have been examined in this program, and judgments might be made with respect to unproductive classes of compounds. Emphasis in synthesis should be placed on conceptually productive areas and on the acquisition of novel molecular structures. This would change the make-up of the input, and of itself, might decrease the total. On the other hand, development of micro-methods for synthesis and molecular identification has increased the number of available small sample compounds, and rejection on the basis of amount offered, especially for novel structures, is contraposed to the screening concept. Thus, the desirable attributes of a primary screen are; predictability for the clinic, feasibility for testing large numbers of limited quantity materials, rapid elimination of inactives, and high cost efficiency so that more resources can be applied to secondary screening and detailed study of "active" drugs.

The possibility of using one or more rapid and relatively inexpensive cell culture, biochemical, or antimicrobial assays for initial screening is being studied. Routinely, in this program, a cell culture (KB) screen is used, along with the in vivo P388 screen, for initial testing of crude natural products. Those showing correlative activity in both are fractionated using the less expensive KB system to bioassay the activity of the fractions. KB is also used to test synthetic materials received in an amount of 45 mg or less and for selected other synthetics of interest. The value of cell culture for bioassay and for identifying highly cytotoxic materials for further biochemical studies and broader spectrum in vivo screening is incontrovertible. Use of this cell culture model as a pre-screen would tend to select a population of compounds that would yield a higher percentage of in vivo actives than de novo in vivo screening. However, a recent retrospective study showed that it would have missed a substantial number of drugs active both in animals and in man. Of 2,022 KB actives that were tested in vivo, 313 (15 percent) were active in at least one in vivo system. However, 451 compounds which were negative against KB showed confirmed activity against at least one animal tumor in vivo, and included Melphalan (NSC-8806), Prednisone (NSC-10023), Hexamethylmelamine (HMM, NSC-13875), Myleran (NSC-750), Cyclophosphamide (CPA, NSC-26271), DTIC (NSC-45388), Procarbazine (NSC-77213), Dibromodulcitol (NSC-104800), Adriamycin (ADR, NSC-123127), Bleomycin (NSC-125066), and the nitrosoureas, CCNU (NSC-79037) and MeCCNU (NSC-95441). Of course, in vitro results, as in vivo results, are dependent on the cell line, experimental method, parameter of growth inhibition, and the quantitative cut-off for "activity". Comprehensive results on activity in other mammalian cell culture screens do not exist for a sufficient number of compounds that have been evaluated in animals or in the clinic to permit such retrospective analyses. Prospective analyses of correlations among alternative cell culture screens, animal screens, and clinical value would require considerable basic and developmental investigation.

Biochemical models are used in this program to study the intracellular actions of drugs which are in the clinic or in development to clinical trial, to study structure-activity relationships within structrual classes of interest, and to determine the degree of similarity of biochemical sites of action among in vivo active structural congeners (see current reports, MBMD Section and Biochemistry Section). Model systems are designed individually to measure actions against precise stages of the cell life cycle, biochemical pathways, or target enzymes. They are useful for determining the ability of a designed compound to elicit the action proposed and, if the compound shows even minimal antitumor selectivity, this could lead to profitable new synthetic approaches. Clearly, the use of any single biochemical end-point for large scale screening would severely restrict the kinds of active compounds detected. A spectrum of broad biochemical screens; e.g., DNA, RNA, and protein inhibition, used as a pre-screen would tend to circumscribe a population of agents containing a relatively high percentage of in vivo actives, but might tend to compromise our ability to detect new antitumor drugs with important, but yet unknown, mechanisms of action. Increasing the number of test systems to include all novel approaches that emerge from basic studies might be most profitable, but would substantially increase screening costs. Antimicrobial screens are used to study patterns of cross-resistance among clinically active drugs and their structural congeners. In addition, drugs in development to the clinic are tested against a spectrum of microorganisms to provide a sensitive tool for determining the in vivo physiological distribution of drug activity. The use of an antimicrobial model as a pre-screen for antitumor activity seems hazardous for a number of reasons including the problem of drug transport across a cell membrane so different from that of mammalian cells. Thus, at this time, there is no reason to dispute the earlier conclusion of Gellhorn and Hirschberg (Cancer Research, Suppl. 3, 1955) that "there is no evidence for the existence of any non-tumor system which could replace a tumor system as a screening tool for carcinostatic agents." This is particularly applicable to the problem of "random" screening where one must test a large number of diverse materials for which there is no prior indication of biological activity either real or imagined. Materials which may be new, but are nevertheless designed on the basis of an experimentally valid rationale present a different question. If these demonstrate the proposed experimental activity in vitro, they should be accorded the broadest possible in vivo testing.

Critical evaluation of a number of transplantable animal tumors has led to our current use of L1210 and P388, respectively for primary screening of random synthetic compounds and crude natural products; L1210 because of its demonstrated sensitivity to most clinically active drugs, and P388 because it is quantitatively more sensitive than L1210 (Venditti, Pharmacological Basis of Cancer Chemotherapy, 1975, p. 245, Williams and Wilkins Co., Baltimore; Goldin et al, Cancer Chemotherapy Reports 50: 173, 1966). If initial screening must be restricted to one system, and presuming that there will be a broadening of the number of systems used for secondary screening to provide a further basis for selection of drugs for priority development, an initial screen of greater sensitivity than L1210 should be considered. P388 appears to be as predictive as L1210 for agents active against human tumors and is more sensitive than L1210 to the clinically useful representatives of most major classes of antitumor drugs. The use of P388 as the initial screen for all materials is currently under consideration, and other models of even greater sensitivity will be investigated. Such a system, in addition to being rapid and relatively inexpensive, should select a high percentage of true positive drugs for the clinic. Selection of a high number of potential false positives would be expected, but not necessarily undesirable if a substantial secondary screening spectrum were used to evaluate them further. Priorities for development could be based on spectrum of activity and on degree of activity in sensitive systems. Pending the possible replacement of L1210 by P388 or some alternative in vivo model, current screening procedures for random synthetic materials, as modified in April, 1975, calls for initial testing against L1210 using the mini-screen approach described in previous reports. Crude natural products will continue to be screened initially against P388 using current protocols (Cancer Chemotherapy Reports, Part 3, 3: 1972) until the current evaluation of the applicability of the mini-screen to natural product screening is completed.

During the past year, the Southern Research Institute (SRI, N01-CM-43756) in re-evaluating the L1210 mini-screen confirmed previous studies at Microbiological Associates (MAI, N01-CM-33728) and Arthur D. Little, Inc. (ADL, N01-CM-33727) showing that the reduction in number of mice per test group from six to three and the restriction on treatment schedule from daily treatment to intermittent treatment did not impair ability to detect materials known to be highly active against L1210. Applicability of the model to large-scale screening of random acquisitions was examined for over 2,000 materials in seven laboratories. Results were compiled, computer processed, and organized by the Value Engineering Co. (N01-CM-33706) and analyzed by the Applied Mathematics and Statistics group at ADL in collaboration with DEB staff. It was concluded that we should use three mouse test groups for initial screening and six mouse test groups for confirmation of activity. The cut-off for activity should be lowered from the current 25 percent increase in lifespan (ILS) to 20 percent in this initial two-step stage of screening. Since we generally recommend for development to the clinic, novel structures achieving a 50 percent ILS against L1210 in subsequent more definitive testing or significant activity in a secondary screen, the impact of this change in initial screening cut-off on expected performance in further testing was analyzed. While it would increase the incidence of false positives (for a true ILS ≧ 50 percent), it would also decrease the incidence of false negatives, our major concern; particularly for daily treatment where the chance of missing a true positive would be negligible. We expect less than 5 percent of the total input to show activity at the 20 percent ILS level, and for these, definitive subsequent evaluation against L1210 (in which a wide range of treatment schedules is used) and against a spectrum of secondary screens will be possible within the current screening capacity. The current mini-screen study showed that daily treatment was more productive and selective than intermittent treatment. However, compound sample size often precludes the use of daily treatment. Therefore, the schedule for the mini-screen will be varied depending on compound availability. The main advantage of the "mini-screen" is that it permits *in vivo* testing with a minimal amount of compound. Also, it decreases animal costs substantially. The decrease in cost per test in the screening laboratory is minimal, but significant when multiplied by the number of tests conducted annually.

Historically, many drugs have entered clinical trial on bases other than the limited primary animal screens that have been used in this program. These have included reports of activity in the clinic, in animal screens from other programs, *in vitro*, in endocrine systems, and in immune response screens. It should not be inferred from this report that alternative biological, biochemical, or pharmacological activities should not form the basis for consideration for clinical trial. However, regardless of the genesis of a new agent, unless it is believed to affect some factor of unique importance to the human host-tumor relationship, its ability to elicit antitumor selectivity or to potentiate the efficacy of an existing therapy in some experimental *in vivo* system should be a prerequisite for its clinical trial. This concept presupposes the widening of the spectrum of *in vivo* models to include diverse transplantable, spontaneous, and virus induced tumors which exhibit different growth, biochemical, histopathological, and immunological characteristics of known or potential importance. For example, neither L1210 nor P388 is very sensitive to hormonal agents or agents designed to enhance immune responses. This program should develop a wide spectrum of such models to be used fully or selectively for the evaluation of compounds emerging as active from its own primary screens and compounds reported from within or outside this program to have interesting and potentially important biological, biochemical, or pharmacological actions. At present, such compounds are studied in detail against L1210 and/or P388 with emphasis on factors known to influence efficacy; e.g., route and treatment schedule, tumor implant site including intracranial, tumor burden at the time of treatment initiation, in combination chemotherapy, etc. In addition, they are screened routinely against the relatively drug resistant B16 and LL tumors and may be screened selectively, depending on existing data, in other highly specialized model systems including drug resistant tumor variants and animal models of specific human diseases.

Routinely, new structural congeners of clinically active drugs are examined in *in vitro* and *in vivo* systems selected on the basis of the characteristics of the parent drug. Objectives of this project, which was conducted at MAI during most of the past year, but is now conducted at ADL are to determine whether the new active congener differs from the parent with respect to its biological, biochemical, or pharmacological action and thus should be considered a unique agent; to provide rapid feed-back of information to synthetic chemists to aid the direction of synthesis; and to determine potential therapeutic superiority over the parent drug.

Secondary screens in current limited use or in development include the Ridgway osteogenic sarcoma (ROS), murine colon tumors and mammary carcinomas, a mouse renal cell carcinoma, and human tumors grown in immunologically deficient mice (see current report, Screening Section, DEB). While none of these is used routinely at present, some have demonstrated their value for the in-depth evaluation of selected individual drugs, drug combinations, and combined modality therapy of clinical interest.

ROS has demonstrated sensitivity to Actinomycin D (NSC-3053), ADR, Daunomycin (NSC-82151), Bleomycin, CPA, Melphalan, 5-Fluorouracil (5-FU, NSC-19893), 6-Mercaptopurine (NSC-755), Vincristine (VCR, NSC-67574), and Cis-Pt-II (NSC-119875). It is insensitive to nitrosoureas and a number of other alkylating agents and antimetabolites. The model permits the evaluation of regression of established tumors, time to regrowth, survival time, and cure rate. It has been an excellent model for the detailed evaluation of drug combinations; e.g., concomitant vs sequential treatment (SRI).

Four transplantable colon tumors showing varying degrees of differentiation have been developed (SRI). The more differentiated of these adenocarcinomas are sensitive to fluorinated pyrimidines, CPA, and nitrosoureas. When the tumor is implanted IP, the liver is the primary site of metastasis. When it is implanted SC, the lung is the primary site of metastasis. Further development for use as a model for surgery plus chemotherapy is being pursued.

Spontaneous mammary carcinoma in CD8F1 mice and first generation transplants of this tumor are used to study drug combinations and combined treatment modalities (Catholic Medical Center, NO1-CM-23703). The tumor is sensitive to CPA, Melphalan, ADR, and 5-FU. The superiority of surgery followed by Melphalan over surgery alone or chemotherapy alone in this model appears to correlate with clinical findings. Studies were extended to show that surgery followed by the combination of Melphalan, ADR, and 5-FU was superior to surgery followed by Melphalan only. The model is especially suited to measuring time to recurrence of primary tumors during drug treatment following surgery and time to development of lung metastases. The group at Mason Research Institute (NO1-CM-57003) has shown the superiority of surgery plus CPA over surgery alone or CPA alone against rat mammary carcinoma 13762. Further development of these and other experimental breast cancer models should be given a high priority during the next year. More emphasis will be placed on early treatment with new drugs because recent development of reliable methods for early diagnosis of human breast tumors (mammography and thermography) raises the possibility that patients will present for treatment with small tumor cell burdens which might be destroyed if more effective drugs became available. The Chester Beatty group (NO1-CM-43736) has progressed in attempts to grow human breast carcinoma in athymic (nude) mice. In addition, we are implementing a project to study the biological characteristics and response to drugs of a premalignant transplantable hyperplastic alveolar nodule in mice. The hyperplastic nodule becomes malignant in a predictable way. The model can be used to measure drug activity against the hyperplastic nodule, against malignant transformation, and against the established tumor.

The Chester Beatty group has shown that HMM, but not CPA can eradicate human lung carcinoma grown in mice made immunologically incompetent by T-lymphocyte depletion. T-cell deprived mice have been used to successfully grow human melanomas and carcinomas of the rectum and ovary as well. The ADL group is beginning investigations of drug effects against mouse lung tumors established after IV implantation of a transplantable squamous cell carcinoma. This system, developed by P. Nettesheim of the Oak Ridge National Laboratory, will be evaluated as a potential model for human lung squamous cell carcinoma.

During the next year, increased emphasis will be placed on the development and use of models for the evaluation of combined treatment modalities. DCT had identified, as a major approach to improving cure rates among human solid tumors the integration of chemotherapy with local modality therapy; i.e., surgery or radiation. A number of ongoing projects within DEB include pertinent tasks. The development of models for evaluating surgery plus chemotherapy were noted earlier in this report. In addition, the ADL group has quantitated the activity of radiation against local B16 tumor, and we plan to extend these studies to LL and other established SC and IM tumors. The group at Washington University (NO1-CM-43713) is studying in vivo the interactions of drug combinations and drugs plus radiation on both malignant cells and normal hematopoietic stem cells. The Allegheny General Hospital group (NO1-CM-43730) is conducting similar studies comparing tumor cells and normal intestinal crypt cells. The University of California (NO1-CM-43791) is exploring biochemical rationales for the use of radiation plus clinically effective drugs. It has been shown, for example, that ADR-induced nuclease degradation of cellular DNA augments radiation damage.

As results of the activities of individual drugs, drug combinations, and combined modalities in diverse laboratory models and from the clinic accumulate, it will be desirable to determine which combinations of primary and secondary in vivo and in vitro screens predict best for the clinic, in general, and for the therapy of particular human cancers. Although previous analyses of correlations between activity in animal screens and clinical usefulness were very encouraging, they were restricted in that clinical data were viewed in terms of categories of results rather than numerical values. DEB, through its contract with ADL, and in collaboration with the Investigational Drug Branch, DCT, has been extending and refining the analysis of data, both animal and human, in order to develop a more accurate picture of the relationship of clinical results to screening. To date clinical results for 30 drugs against 17 human solid tumors have been reviewed. For each tumor-compound combination, all available clinical studies were analyzed with regard to number of "evaluable" patients, and number responding. These evaluations reflect response rates, the parameters of response, and the clinicians' consensus judgment regarding "evaluability" and "activity". Response rates have been tabulated for individual human tumor types to show prior therapy, concomitant therapy, regimen, and duration of response. During the next year, more emphasis will be placed on correlations between activity in individual experimental models and usefulness against specific human tumors. Hopefully, on the basis of more quantitative information, both clinical and animal, a multivariate discriminant function, previously developed for establishing priorities for development of compounds to clinical trial, will be refined as a prediction formula. This method provides for the utilization of quantitative results from a spectrum of laboratory models in concert weighing each according to its own demonstrated value, and it will permit the inclusion, into the overall evaluation, results from new in vivo or in vitro systems along with results from established systems.

Within the past year, DEB has initiated a number of new contracts devoted to fundamental aspects of drug action and continued its related intramural studies. Specifically, these objectives are aimed at drug effects on growth kinetics of normal and tumor cells, in vivo and in vitro, and elucidation of cellular sites of action of drugs in development to clinical trial or of special interest. Contract activities related to these tasks are described in the current reports of MBMD Section and Biochemistry Section, DEB. Major findings for 17 drugs have been reported to DCT Decision Point groups and to clinical working groups.

In intramural studies, ten drugs were evaluated for effects on cell cycle progression. Studies in collaboration with Dr. Bhuyan (Upjohn Co. N01-CM-43753) related the cell killing effect of the combination of Cytosine arabinoside (NSC-63878) and 5-FU to the ability of 5-FU to increase the size of the S-phase population. Collaborative studies with Dr. Wolpert-Defilippes (Drug Metabolism Section) showed that Maytansine (NSC-153858) arrested cell progression through M-phase, but allows DNA synthesis without cell division. Human ovarian carcinoma cells, maintained in long term culture were shown to retain differentiated organizational patterns and hormonal production. The development of such human cell lines in culture provides a further basis for the selection of individualized chemotherapy.

Table 1

Contracts Monitored in Whole or in Part by DEB Staff, FY 75

Number	Institution		Number	Institution	
	(Contracts under direct DEB management)*				
43730	Allegheny General Hospital	(M)	02225	Miami, University of	(S)
10056	Atomic Energy Commission	(M)	53789	Michigan Cancer Foundation	(M)
33727	Arthur D. Little, Inc.	(C)	43785	N.Y. Dept. Health, Health Res.	(M)
53765	Arthur D. Little, Inc.	(C)	43788	Minnesota, University of	(M)
57006	Arthur D. Little, Inc.	(S)	53830	Pennsylvania, University of	(M)
57005	Battelle Mem. Inst. Columbus	(S)	22042	Scripps Clinic and Res. Foundation	(B)
57008	Battelle Mem. Inst. Frankfurt	(S)	43756	Southern Research Institute	(C)
43790	Brigham Young University	(M)	43784	Southern Research Institute	(M)
43791	California, University of	(M)	43786	Texas, University of	(M)
23703	Catholic Medical Center New York	(S)	33706	Value Engineering Co.	(A)
53825	Harvard University	(M)	53821	Value Engineering Co.	(A)
57007	Hazelton Laboratories	(S)	53770	WARF Inst. Inc.	(S)
43755	IIT Research Institute	(S)	43731	Washington University St. Louis	(M)
57004	IIT Research Institute	(S)	33733	Wisconsin, University of	(S)
PL480	Indian Cancer Res. Centre	(S)	12339	Yale University School of Medicine	(B)
33720	Inst. Pharmacol. Res. Mario Negri	(S)	33711	Yale University School of Medicine	(B)
33741	Litton Bionetics, Inc.	(C)	53824	Yale University School of Medicine	(B)
53792	Madrid, University of	(M)	43787	Yeshiva University	(M)
57003	Mason Research Institute	(S)			
	(Contracts managed by other DCT units with part of effort monitored by DEB)+				
43736	Chester Beatty Research Inst.	(DDB)	53849	Arthur D. Little, Inc.	(EXT)
53840	Institute Jules Bordet	(LEC)	22054	Japanese Fd. Cancer Research	(LEC)
33702	Michigan Dept. of Health	(DDB)	33728	Microbiological Associates Inc.	(DR&D)
92019	Research Triangle Institute	(DDB)	33750	Arizona, University of	(DDB)
43753	Upjohn Company	(DDB)	02083	Southern Research Institute	(EXT)

*DEB - Office of the Chief (C), Automated Information Section (A), Biochemistry Section (B), Molecular Biology and Methods Development Section (M), Screening Section (S).

+DDB - Drug Development Branch, LEC - Laboratory of Experimental Chemotherapy, DR&D - Office of the Associate Director, EXT - Experimental Therapeutics, DCT.

Table 2

Distribution of DEB Monitored Contract Funds by Project Area and DCT Array

Stage	Project Description	DCT Array	Amount (Dollars)
I-1XX	Organic synthesis directly related to in-house experimentation	LA-	102,000
	Collection of synthetic compounds for screening	LA-	25,000
	Bioassay of natural products *in vitro*	LA-	123,000
	Related new model development	EA-	24,000
	Bioassay of natural products *in vivo*	LA-	702,000
II-2A1	Primary screening *in vitro*	LA-	321,000
	Secondary screening *in vitro*	SA-	57,000
	Related new model development	EA-	69,000
	Primary screening *in vivo*	LA-	2,616,000
	Secondary screening *in vivo*	SA-	233,000
	Related new model development	EA-	370,000
II-2A2	Cell kinetics and biochemistry	SA-	135,000
	Molecular biology	EA-	38,000
II-2B2	Detailed drug evaluation *in vitro* including biochemical mechanisms of drug action	SA-	878,000
	Related new model development	EA-	269,000
	Detailed drug evaluation *in vivo*	LA-	1,662,000
	" " " " "	SA-	828,000
	Related new model development	EA-	483,000
III-3X1	Pharmacology and Pharmacokinetics	SA-	18,000
III-3X2	Combination chemotherapy	SA-	775,000
	Combined modality therapy		
	Chemotherapy plus immunotherapy	SA-	6,000
	Chemotherapy plus radiation	SA-	199,000
	Chemotherapy plus surgery	SA-	126,000

Table 2, Cont'd.

Distribution of DEB Monitored Contract Funds by Project Area and DCT-Array

Stage	Project Description	DCT Array	Amount (Dollars)
-----	Support Activities		
	Applied mathematics and statistics	LA-	150,000
	Frozen tumor and cell bank	LA-	8,000
	Service to monitor screening projects	LA-	213,000
	Data processing and retrieval	LA-	830,000
	Clinical drug distribution inventory	LA-	43,000

Array Code	Linear LA	Supplementary SA	Exploratory EA	Total
I-1XX	952,000	0	24,000	976,000
II-2A1	2,937,000	290,000	439,000	3,666,000
II-2A2	0	135,000	38,000	173,000
II-2B2	1,662,000	1,706,000	752,000	4,120,000
III-3X1	0	18,000	0	18,000
III-3X2	0	1,106,000	0	1,106,000
Support Activities	1,244,000	0	0	1,244,000
Total	6,795,000	3,255,000	1,253,000	11,244,000

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AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to DEB, Drug Research & Development (DR&D). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories subsequent to entry into the system. By means of the biological activity or inactivity reported, decisions are made concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of being queried by special programs thus providing special summary data for : 1) evaluation of the existing screens; 2) design and evaluation of new test systems; 3) review of compounds being considered for Division of Cancer Treatment (DCT) Decision Point 2A; 4) further evaluation of individual compounds and drug combinations; 5) statistical information required for effective program management.

AIS is staffed by two professionals, one statistical assistant, one computer aide, one Project STRIDE trainee, and one secretary. Professional staff serves as Project Officers and has responsibility for two contracts: Value Engineering Co. (NO1-CM-33706) which provides the major service for the biological data processing system, and Value Engineering Co. (NO1-CM-53821) which maintains the DCT clinical drug distribution and inventory system. Also, during the past year, an increased portion of staff time was devoted to providing special reports (queries) to DCT staff members, contractors, and suppliers. The number of such queries increased from 75 (in 1973) to 142 for calendar year 1974. AIS staff has continuously upgraded the schedule dependency summary and plotting programs and files.

The current contract (NO1-CM-33706) for the operation and maintenance of the biological data processing system has been in effect since July 1972. During 1974, 13,758 experiments, which include 400,463 single drug tests, and 18,551 drug combination tests from 630 combination chemotherapy experiments were processed, an increase of 4% over 1973 (Table 1). A total of 37,821 materials were added to the master files, a decrease of 14% from 1973. Costs (both contractor and Division of Computer Research and Technology) increased from $1.90 per test line in 1973 to $2.01 in 1974. This was due primarily to the increased cost of paper and forms. It should be noted, however, that the cost per test line in 1972 was $2.15 and that efficiencies implemented by the contractor reduced this cost in 1975. A significant effort was devoted to setting up and processing a test series identified by a Special Study Code of M. This testing, necessary for definitive evaluation of the "mini-screen" provided data for statistical analysis of synthetic testing to determine the effect of the number of animals per test group and the treatment schedule on yield of true active compounds. Tasks included preparation of the special programs and procedures to support this testing, setting up special files including a tape in a format containing data unique to this system, and preparation of required special reports.

Improvements were made to the Combination Chemotherapy programs. These included increasing the number of drugs allowed in combination to more than two, and revision of output for each single drug to show the existence of combination treatment data for that drug.

Programming required to automatically assign Test Status Codes (TSC) and report instances where the TSC assigned by the screener differed from that assigned by the program was completed in 1974. At the end of the year, the computer assigned TSC was being used in Special M. Code processing with the value assigned by the screener still being used for other data.

New reports have been added for management use including: 1) a report which increases the significance of certain data from the Natural Products data processing system; 2) a report which helps screening laboratories determine the source of errors introduced into the system and 3) several special reports which aided in the analysis of data from the Special M. study. Modifications to reports involved increasing the number of lines printed per page on four large reports thereby reducing the amount of paper required per printing. This reduced the cost per print by using less paper, and required fewer lines being printed (another cost reduction). In turn this lead to fewer pages being reproduced. In keeping with the progress made last year, the use of microfiche as a query output increased.

During 1974, significant progress was made on the Front End Redesign. By the end of the year, the editing and error handling portion of the DEB Biological Data Processing System had been reprogrammed in a modular system, and the basic design for the other modules in the new system was complete. Programming and analysis was underway on calculations redesign, input/output handling, error analysis routines, the Combination Chemotherapy system and management information reports. The Front End Redesign will greatly enhance the flexibility of the system and therefore improve the overall efficiency of the processing system. This will be accomplished by the fall of 1975. This present year will be focused primarily upon current production needs, and implementation of the redesigned Front End. With the forecast that the Natural Products subsystem will have an increase in the amount of data to be handled, an analysis of that system is in order. At present, this subsystem is cumbersome and as a result is comparatively inefficient to operate. The projects outlined above will be carried out within the current operating budget. However, we envision the need for analysis and programming in other aspects of the biological data processing system to facilitate responses to requests for data not currently processed and changes in the program to accommodate new experimental assay.

The Clinical Drug Distribution & Inventory System was maintained and revised under contract (NO1-CM-53821). Daily, monthly, and quarterly reports were prepared on schedule, and over 90 special query reports were produced. Many programs were rewritten and new report formats designed, in order to maintain the efficiency of the system as the number of clinical drug shipments increased.

TABLE 1

DIVISION OF CANCER TREATMENT PROGRAM STATISTICS

	Calendar Year 1974	Calendar Year 1973	Calendar Year 1972	Calendar Year 1971
1. Materials Added to the Master Files*				
Synthetic Compounds	28,644	26,906	14,781	9,066
Selected Agents	107	214	-	-
Plants	5,043	11,066	10,434	2,825
Fermentation Products	677	2,550	2,712	1,215
Animal Products	1,597	3,034	1,669	1,120
Total	36,068	43,770	29,596	14,226
2. Tests Performed				
L1210 Lymphoid Leukemia	211,832**	134,562	103,681	102,424
P388 Lymphocytic Leukemia	124,396**	198,723	180,027	53,449
Walker Carcinosarcoma	909	1,704	2,770	4,368
B16 Melanoma	11,724	15,989	6,028	1,423
Lewis Lung Carcinoma	2,770	2,441	4,141	2,803
AK Leukemia	925	-	-	700
Other Tumors	2,821	2,696	6,458	1,080
Total In Vivo (Excluding Toxicity)	355,377	356,115	303,105	166,247
Toxicity	9,215	6,430	2,900	2,835
Cell Culture	35,371	31,820	25,996	12,485
Alkaloid	500	450	-	-
Biochemical Assay	-	337	231	392
Total Non In Vivo (Including Toxicity)	45,086	39,037	29,127	15,712
Total of ALL tests	400,463	395,152	332,232	181,959

* These figures are derived from the Drug Research and Development Biological Data Processing System and differ from those generated by the Drug Development Branch, which logs in materials when NSC numbers are assigned. A material first enters the data processing system when its receipt is acknowledged by the screener which may be two to four months later. ** Includes tests against Resistant Tumor Lines; 970 in L1210 and 431 in P388.

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The ultimate objective of the Biochemistry Section program is the improved therapy of human cancer by drugs. The program is implemented through contracts with qualified institutions and through informal, unfunded arrangements with individual investigators, usually at universities, who have produced agents of theoretically exploitable qualities which require special testing to demonstrate anticancer activity, or who possess mastery of particular techniques and procedures needed for definitive work on DR&D drugs. In addition analog testing is also supervised by this section. The following describes first the work being done under contract, and next that under the informal arrangements.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CM-22042)

This contract, which has been in effect for about four and one half years, is to terminate April 15, 1975, at the request of the Principal Investigator. Its objective was to prepare analogs of vitamin B_{12} and evaluate them as antitumor agents. Synthesis of agents, by combined enzymatic and chemical means, was readily performed. Evaluation by inhibition of ribonucleotide reductase from Lactobacillus leichmannii was straightforward. However, evaluations progressing through mammalian cell B_{12}-requiring enzymes to in vitro cytotoxicity and in vivo antitumor activity presented unexpected difficulties and uncovered gaps in fundamental knowledge of the specific definitive role of this vitamin in mammalian cell metabolism and division. More basic studies will be necessary before this metabolic area becomes ripe for exploitation. Accordingly, this work is in the process of being transferred to grant support through the usual channels.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-12339)

The purpose of this contract is to produce new useful anticancer agents which have their primary effects on the production or processing of various nucleic acids. To accomplish this goal, acridine, known to intercalate into nucleic acid but with weak binding and little or no antitumor activity, has been used as a model for modification. From deductive reasoning, derivatives have been prepared, and their effects on physical and biological parameters determined. These data have led to the synthesis and assessment of effects of still other changes in the molecule. The current "best" agents are a series of diacridines, i.e. two 9-amino-acridine molecules connected through the amino groups by $-(CH_2)_n-$ chains, where n=2 through 18. Through both space-filling models and electric dichroism data, these agents were found to fall into three distinct classes, each of which has specific biological effects, producing, in essence, three different types of agents: (1) n=2. This is a single intercalator with the unintercalated ring held parallel without free rotation. (2) n=3,4,6. The unintercalated ring of these single intercalators is free to rotate.

(3) n=8,10,12. These compounds are double intercalators; n=8 can fit on either side of a base pair, while n=12 could form a two-base pair sandwich.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-33711)

Screening of DR&D-acquired antifols for inhibition of dihydrofolate reductase (DHFR) continues under this contract. Among a group of 14 quinazolines sent directly by Parke-Davis, all were in the strong inhibitor range, four especially so.

Preclinical pharmacology of the Baker antifol (NSC-139105) was completed and published this year. Clinically, cerebrospinal concentration of NSC-139105 was found to be 2% or less of serum levels, but the drug may be concentrated in brain. One patient had simultaneous levels in serum 1.2×10^{-5}M, CSF 1.8×10^{-8}M, brain 3×10^{-4}M.

Comparison of leucovorin reversal of inhibition of DNA synthesis by MTX or NSC-139105 (Baker antifol) in normal human marrow cells from two patients showed complete reversal of MTX effects, but variably incomplete reversal of NSC-139105 inhibition which itself varied. The variability of DNA inhibition by NSC-139105 correlates with clinical data where hematological toxicity occurs in some but not all patients at the same dose.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM-53824)

The purposes of this contract are to synthesize agents which can interfere with cell membrane formation or function and to evaluate them in various *in vitro* and *in vivo* systems. The contract, which was activated in late November 1974, is still essentially in the start-up phase. The first agents selected for preparation are 2-deoxy-2-fluoro-L-fucose and -L-rhamnose. Their synthesis is partially completed.

G. A. LePAGE, UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA, CANADA

The association with Dr. LePage is a voluntary one involving no funds. Agents of mutual interest are discussed with him, and if he has the time to study them, DR&D supplies the agents. During the past year, two significant contributions have come from Dr. LePage: (1) a study of the Parke-Davis adenosine deaminase (NSC-218321D) and (2) clear identification of selenium guanosines as a class of agents having a different mechanism of action than thioguanosines.

DR. KARL FOLKERS, UNIVERSITY OF TEXAS, AUSTIN, TEXAS

Dr. Folkers believes that coenzyme-Q is higher in many tumors than in normal tissues, and that analogs of coenzyme-Q should have a selectively toxic effect on tumors. The agents he makes are screened by him in an *in vitro* test for inhibition of mitochondrial electron flow. Only active agents are sent to us for *in vivo* tests. Of about 20 coenzyme-Q analogs which Dr. Folkers has made in sufficient quantities for *in vivo* testing, none has shown activity in P388 or, when tested, in L1210. However, i.p. Walker 256 appears to be a suitable test system to discriminate among these analogs. It remains to be seen whether Dr. Folkers will be able to produce enough different agents in sufficient quantity to allow the selection for clinical trial of a promising agent with this interesting mechanism of action.

ANALOG TESTING

This activity has only recently been transferred to Arthur D. Little, Inc. (N01-CM-53765) and the Biochemistry Section. Protocols for parent-analog comparative testing were worked out by the Analog Subcommittee of the Drug Evaluation Committee for each of the major types of agents for which DDB, DR&D has contracted production. In addition, expedited testing and reporting to supplier of all other contract-generated agents and intermediates, except natural products, are done under two protocols, "nucleoside" and "regular". Seventy three percent of the expected agents fall into the last two classes. This project in the past has been primarily a service to contract suppliers to guide their synthesis programs. It is hoped that, while retaining the service function, analysis of the data in the future will serve as a basis to evaluate the present effectiveness of the analog synthesis program to DR&D objectives.

Analog screening should be the means of identifying portions of the parent molecule which are inviolable and those subject to modification. But active analogs, after this initial exploration, should ultimately be tested for the degree to which they achieve the objective of their synthesis, i.e. cardiotoxicity for anthracyclines and nephrotoxicity for platinums. Only these two models now exist. More should be developed. Ideally the whole process of analog procurement and testing should be integrated and planned at one time. First should be a definition of objectives (clinicians should certainly have an input here), then (unless the aim is H_2O-solubility, or production) the models for definitive testing should be developed, and finally the production of agents.

FUTURE PLANS

There are two additional projects which are being undertaken by the section. The first is the one-time task of identifying for further study agents acquired in the past, the antitumor activity of which at the time they were tested fell below current criteria for recommending them for clinical trial.

The second task is a long-term and slow-yielding one. It is to identify among active analogs of well-known agents, those which possess some important property which would make them worthy clinical candidates. In dealing with this problem many aspects need to be considered, and each series of drugs will need to be dealt with in a different fashion. Varying scientific expertise will also be required, sometimes to be found in contracts within the section and branch, but often extending to those in other parts of DCT or to new resources. Governing all selection of classes of agents to receive this kind of work would be clinical interest and/or clinical problems.

MOLECULAR BIOLOGY AND METHODS DEVELOPMENT SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Molecular Biology and Methods Development Section (MBMD) is staffed by five senior professionals (including two commissioned officers, one senior staff fellow and one visiting fellow) and three technical and clerical employees. The principal objective of this section is to conduct basic and developmental research leading to the establishment of new and improved biological and biochemical drug evaluation assays by coordinating both intra-mural and contract supported laboratory programs. Specifically, these objectives are to be achieved by pursuing fundamental aspects of (a) the growth kinetics of tumor cells and normal cells *in vivo* and *in vitro* (b) the effects of potential antitumor drugs on the growth kinetics of tumor cells and normal cells *in vivo* and *in vitro*, (c) identification and characterization of drug-receptor site interactions, (d) the development of new *in vivo* and *in vitro* screens of greater predictive value for detecting agents with specific human tumor activity and (e) the development of rational bases for the prediction of optimally effective combined modalities of treatment and treatment schedules.

Significant Accomplishments

Direct Contract Activities

The section has primary or significant responsibility for 17 research contract projects designed to achieve its objectives. These contracts, their objectives and achievements are listed below. Most of them are now in their first year of funding.

Southern Research Institute (N01-CM-43784) $347,862. Objective: This contract has five separate but interrelated objectives: (a)to determine if new antitumor drugs have biochemical activities similar to clinically established drugs (b) to provide clear leads to biologically significant biochemical properties of new antitumor drugs, (c) to provide details, for selected drugs, of effects on *de novo* purine and pyrimidine biosynthesis, (d) to develop systems to select structural analogs having more desirable biochemical properties and, (e) to develop biochemical rationales for the combined use of clinically established antitumor drugs. Major Findings: The detailed findings are numerous. All aspects of these objectives are proceeding well but are still in the early stages of development. Significance: To provide supplementary information to guide the development of potential therapeutic drugs and for the more effective use of clinical drugs. Proposed Course: To continue the current project objectives.

Seven other contracts have in common, the objective of providing detailed biochemical characterizations of antitumor drugs. They differ in the particular area of biochemical expertise. Their common significance to the Division of Cancer Treatment program is to provide the possibility of rational approaches to analog development, therapeutic applications and drug combinations.

University of Texas (N01-CM-43786) $80,226. Objective: Details of effects on polynucleotide metabolism. Major Findings: A preliminary evaluation of Acronycine (NSC-403169), Hycanthone (NSC-142982), Galactitol diepoxide (NSC-132313), Cain's Acridine (NSC-141549), DL-Alanosine (NSC-153353), Ellipticine (NSC-71795), Quinolinium (NSC-176319), and Inosine diglycoladehyde (NSC-118994) has been completed. Proposed Course: To proceed to detailed studies of the effects of some of these drugs on polynucleotide metabolism.

Yeshiva University (N01-CM-43787) $82,797. Objective: Details of effects on protein synthesis. Major Findings: The effects of Harringtonine (NSC-124147), Anguidine (NSC-141537), and Bruceantin (NSC-165563) on protein synthesis were evaluated in detail and found to have significant actions. The effects of Bleomycin (NSC-125066) and VP-16-213 (NSC-141540) on DNA structure were also characterized. Proposed Course: To continue to evaluate drugs being developed for clinical trials and drugs already in clinical trials.

Brigham Young University (N01-CM-43790) $69,254. Objective: Details of effects on folate metabolism. Major Findings: To date the major emphasis has been on providing the enzyme systems in which candidate drugs will be studied. The purification of serine transhydroxymethylase, N-5,N-10 methylene tetrahydrofolate dehydrogenase and methionine synthetase is underway. Proposed Course: To evaluate the effects of known anti-folate therapeutic drugs on most of the enzymes of mammalian folic acid metabolism.

University of Madrid (N01-CM-53792) $28,400. Objective: Effects on electron transport processes in aerobic energy metabolism. Major Findings: The most active drugs as inhibitors of mitochrondrial electron transport are Baker's Soluble Antifol (NSC-139105), Dichlorolawsone (NSC-126771) Tritylcysteine (NSC-83265), VM-26 (NSC-122819), and Ellipticine.. Adriamycin (NSC-123127) was found to be an effective inhibitor of Na-K dependent ATPase. Proposed Course: To study the effects of Adriamycin and its analogs on Na-K dependent ATPase in greater detail to evaluate its role in their therapeutic and toxic properties.

University of Minnesota (N01-CM-43788) $65,336. Objectives: Mechanisms of drug uptake and effects on membrane transport processes. Major Findings: A major part of this contract has been devoted to a study of the mechanism of cell uptake of Inosine diglycolaldehyde and its effect on other cell transport processes. Other drugs under study are DL-Alanosine and purine and pyrimidine analogs. Proposed Course: To continue the study of these and other therapeutic drugs.

Michigan Cancer Foundation (N01-CM-53789) $55,089. Objective: Details of effects on membrane structure and properties. Major Findings: Tritylcysteine was found to significantly alter cell membrane permeability to Actinomycin D (NSC-3053). Some members of a series of its analogs were found to be even more active. Proposed Course: More details studies on the membrane effects of Tritylcysteine will be done using its radioisotopic form.

University of Pennsylvania (N01-CM-53830) $21,422. Objective: To investigate the bioenergetic aspects of cancer chemotherapeutic agents. Major Findings: Systems are being developed to evaluate the acute and chronic effects of drugs on the energy performance status of isolated perfused organs. The effects of Adriamycin on the energy performance status of the isolated heart, are being studied. Proposed Course: To continue this project as it has been initiated.

Four of the contract projects are designed to provide special biochemical information of potential use to drug development.

University of California (N01-CM-43791) $90,971. Objective: To provide biochemical rationales for the combined use of radiotherapy and clinically established antitumor drugs. Major Findings: The combined action of x-radiation and Cyclophosphamide (NSC-26271), Hydroperoxycyclophosphamide and Bleomycin (NSC-125066) have been studied. Evidence suggests that Adriamycin causes nuclease degradation of cellular DNA and augments x-radiation induced damage. Significance: To provide a rational basis for the selection and interpretation of drug-radiation protocols for clinical trials. Proposed Course: To continue the current effort.

Harvard University (N01-CM-53825) $52,376. Objective: To study the cell surface effects of antitumor sugar analogs. Major Findings: This is a newly funded contract. Work has just begun. Significance: This project is based on an exploration of the possibility that an effective chemotherapeutic approach may be based on increasing cell immunogenicity by an alteration of cell surface polysaccharide composition. Proposed Course: To initiate the contract objectives.

New York State Department of Health (N01-CM-43785) $54,851. Objective: To develop an *in vitro* cell growth profile of drug interactions. Major Findings: A library of drug interactions (antagonistic, additive, synergistic) among 32 drugs of clinical interest is being generated. Significance: The "interaction profile" of a new drug with a test panel of drugs may provide evidence for identifying the action of the new drug as similar to that of a known drug, or for selecting from several structural analogs, those having a different mechanism of action. Proposed Course:

To complete the library and test the system for validity.

Litton Bionetics (N01-CM-33741) $317,699. Objective: Screening of compounds for anti-enzymatic, anti-viral, and antitumor activity. Major Findings: To date, 280 compounds have been tested for cytotoxicity for 3T3 cells, inhibition of transformation by murine sarcoma virus in 3T3 cells, and inhibition of simian sarcoma virus DNA polymerase, mouse embryo DNA polymerase I and II, DNA-dependent RNA polymerases I and II, and poly(A) polymerase. Significance: This screen may be of relevance to both the detection of new potential therapeutic drugs as well as to providing an understanding of the mode of action of known therapeutic drugs. Proposed Course: To deemphasize the screening for enzyme inhibition and to implement systems for the detection of other potentially relevant biological phenomena.

Five contract projects have as their common objective to provide information on the details of effects of drugs on cell biology and growth kinetics. These include both cell culture and *in vivo* systems.

Washington University (N01-CM-43713) $102,493. Objective: To study the interaction *in vivo* of anticancer agents at the cellular level by measuring the survival of normal hematopoietic stem cells and of malignant cells. Major Findings: Dose-survival curves have been obtained with Adriamycin, Cyclophosphamide 5-Fluorouracil (NSC-19893), L-Phenylalanine mustard (NSC-8806), Vincristine (NSC-67574), and x-radiation. With the exception of x-radiation, most of these agents have little effect on leukemic cells. Significance: To provide rationales for optimal drug dose intervals and for drug combination schedules. Proposed Course: To continue and extend these studies to drug combinations.

Allegheny General Hospital (N01-CM-43730) $79,305. Objective: To determine the biological effects of chemotherapeutic drugs on *in vivo* cell populations, normal and malignant. Major Findings: Adriamycin and Cytosine arabinoside (NSC-63878) have been studied for their relative cytocidal effects on gastrointestinal tract crypt cells and the P815X2 tumor, *in vivo*. BCNU (NSC-409962), Adriamycin, Cyclophosphamide, Cytosine arabinoside, and 5-Fluorouracil have been studied for effects *in vitro* and *in vivo* on the P815X2 tumor. Significance: To determine principles for optimal drug dose intervals and for drug combination schedules. Proposed Course: Protocols for evaluating two drug combination schedules based on single drug data (BCNU and Cytoxan, BCNU and Adriamycin, Vincristine and 5-Fluorouracil) are being developed.

Atomic Energy Commission (Y01-CM-10056) $24,500. Objective: To determine the cell cycle phase specific progression and cytocidal action of clinical drugs and of drugs in development for clinical trials. Major Findings: A careful comparison of CCNU (NSC-77037), MeCCNU (NSC-95441), BCNU (NSC-409962), Chlorozotocin (NSC-178248) and Streptozotocin (NSC-85998) was completed and published. Significance: Such information is useful in selecting drug schedules, drug combinations, and in setting priorities for drug development. Proposed Course: The next set of drugs planned for this study is Methotrexate (NSC-740), 5-Fluorouracil (NSC-19893), and Adriamycin (NSC-123127).

Two contract projects, which are not the direct responsibility of this section, have components which support its cell biology objectives. One, Atomic Energy Commission (Y01-CM4-0102), in addition to providing the NCI with a Los Alamos flow microfluorometer and cell sorter, is involved in developing methods for solid tumor sample preparation and in studying experimental tumor cell populations for tumor age dependent changes in cell DNA content distributions. The second, University of Texas (N01-CM-61156), is a pharmacology project. One component of this project is the evaluation of drugs in vitro, on cell cycle phase progression and cell cycle phase cytocidal properties.

Workshops, Symposia, and Reports

Staff members of MBMD were responsible for organizing and presenting a segment of the 1974 N.I.H. Symposium and Exhibit on Recent Developments in Research Methods and Instrumentation: Electronic Cell Sorting. At this meeting the current instrumentation for electronic cell sorting was reviewed and exhibited. This field has a direct application to the analysis of tumor cell proliferative kinetics.

Staff members of MBMD have written reports, "Cell Biology and Biochemistry Data Sheets", on 17 drugs of high priority to the DCT drug development and evaluation program for presentation to Decision Point and Clinical working groups.

Intra-mural Research Projects

Two individual research projects have been conducted within our intramural laboratories. One project(Project No. Z01-CM-03579-02-DEB) titled, "Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression" is oriented to the development of flow microfluorometric techniques and their application to the in vitro characterization of therapeutic drugs. In addition, an empirical approach using this methodology is being taken to develop an in vitro system for identifying drugs with potential therapeutic activity. The second project (Project No. Z01-CM-03583-04-DEB), titled, "Modification of Cell Surfaces to Limit Cell Division and Increase Immunogenicity" is aimed at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells. Additional objectives of this project are to find agents which selectively alter cell immunogenicity and to establishing human tumor cell lines in culture.

Publications: See current report, Drug Evaluation Branch, Office of the Chief.

Project No. Z01-CM-03579-02
1. Division of Cancer
   Treatment, NCI
2. Drug Evaluation Branch
3. Molecular Biology and
   Methods Development
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression.

Previous Serial Number: NCI-3579

Principal Investigators: Vincent H. Bono, Jr. and Robert L. Dion

Co-Principal Investigator: David Abraham

Cooperating Units: The Upjohn Company, Kalamazoo, Michigan, Contract N01-CM-43753

Laboratory of Statistical and Mathematical Methodology, DCRT, NIH

Drug Metabolism Section, LCHPH, EXT, DCT, NCI

Clinical Drug Distribution Section, DDB, DR&D, DCT, NCI

Man Years:

Total	3
Professional	1 1/2
Other	1 3/4

Project Description:

This project is concerned with the development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and for their application to (a) the characterization of therapeutic drugs and (b) the identification of agents with potential therapeutic activity. The major methods employed in this project are (a) mammalian cell culture techniques applied to the growth of L1210 leukemia cells in suspension culture, (b) flow microfluorometric determination of cell DNA content of fluorescent-Feulgen stained cells in a Cytofluorograph (Biophysics Systems, Inc.) and (c) processing, analyzing and plotting cell DNA content frequency distributions by computer.

During the current year, 10 drugs of primary interest were evaluated for their effects on L1210 cell cycle progression: (±)ICRF-159 (NSC-129943), R(-)ICRF-159 (NSC-169779), S(+)ICRF-159 (NSC-169780), Aminothiadiazole (NSC-4728), Piperazinedione (NSC-135758), D,L-Alanosine (NSC-143647), dimethyltriazino imidazolecarboxamide (NSC-45388, DTIC), disodium hexachloroiridate (NSC-247469), Maytansine (NSC-153858), and Daunomycin (NSC-82151). Detailed studies in collaboration with Dr. Bhuyan, The Upjohn Company, showed a correlation between the cell killing effects of combinations of Cytosine arabinoside (NSC-63878) and 5-Fluorouracil (NSC-19893, FU) and the effectiveness of FU in increasing the size of the S-phase cell population. Collaborative studies with Dr. Wolpert, Drug Metabolism Section, showed that Maytansine causes an arrest of cell progression through mitosis and allows reinitiation of DNA synthesis without cell division, resulting in endoreduplication. In collaboration with Dr. Mossiman, DCRT, NIH, an empirical approach to the analysis of the cell DNA content frequency distribution curves is continuing. By the use of linear regression analysis a method for distinguishing therapeutically active drugs from cytotoxic but therapeutically inactive drugs is being developed.

In collaboration with the Clinical Drug Distribution Section, the in vivo therapeutic activity of newly formulated drugs of special interest, e.g., Acronycine (NSC-403169), are evaluated in both standard and special tumor systems.

This project relates to the Drug Evaluation program, DCT in providing a characterization of the cell biological properties of therapeutically active drugs and in exploring the possibility of developing a new screen for the selection of drugs with therapeutic activity. In addition to continuing the current research effort, we plan to measure the DNA content and DNA synthesis rate of individual cells simultaneously.

KEYWORDS: Cancer Chemotherapeutic drugs, Cell cycle progression, cell DNA content, Cytotoxicity, Endoreduplication, Flow microfluorometry, Fluorescent-Feulgen staining, in vitro.

Honors and Awards: None

Publications: See current report, Drug Evaluation Branch, Office of the Chief.

Project No. Z01-CM-03583-04
1. Division of Cancer
   Treatment, NCI
2. Drug Evaluation Branch
3. Molecular Biology and
   Methods Development
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modification of Cell Surfaces to Limit Cell Division and Increase Immunogenicity

Previous Serial Number: NCI-3583

Principal Investigator: Muriel Lippman

Co-Principal Investigator: None

Cooperating Units: Southern Research Institute (W.R. Laster and J. Mayo), National Institute of Child Health and Human Development (Dr. L. Loriaux), Dr. G. L. Gold (Silver Spring, Maryland)

Man Years:

Total	2 1/4
Professional	1 3/4
Other	1/2

Project Description:

Objectives: 1. The project aims at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells.

2. In the case of manifest cancer, the immune response evoked by the malignant cells has failed to effect a complete rejection of the neoplastic cells. Studies are carried out with the aim of increasing the immunogenicity of the neoplastic cells and preventing some aspects of enhancement.

3. Application of cell surface molecules to cultured human cells is immediately relevant to clinical chemotherapy. To this end efforts were made to maintain human tumors in long term culture.

Methods Employed:

Standard procedures for testing drugs on cultured cells and in animals. Nude mice are used for the growth of human cells.

Major Findings: 1. Macromomycin (NSC-170105) (MCR) was found to be curative in Lewis lung under specified regimens and routes of administration. The drug reduced the size of the primary tumor and appears to prevent metastasis. The drug was also shown to increase the immunogenicity of cultured cells resulting in doubling of survival time when such treated cells were injected into animals. Treated cells evoked a cell mediated response which was specific and did not cross react with untreated cells. Purified preparations of MCR from Japan were shown to be up to 200 times as active initially as the impure Bristol preparations but were unstable and lost activity in a matter of 2 months.

2. Human ovarian carcinoma cells obtained by paracentesis were maintained in long term culture. They retained differentiated organizational patterns and hormonal production for at least eight months. Chemotherapy was individualized based on the response of these cells in culture.

Significance for the Program: The Program has need of finding agents with novel modes of action. In 1971 and subsequently, I proposed that the cell surface would be a suitable target for chemotherapeutic activity. The behavior of MCR in culture and in mice shows it to be representative of such a new class of agents.

Proposed Course: 1. Further study on purified MCR activity.

2. The use of nude mice as a tool for evaluation of cytotoxic vs. immune involvement of MCR and other agents.

3. Study of TA3Ha and Ta3Kl tumor systems in culture and *in vivo* as model systems for measure of cytotoxic activity, modified immunogenicity, immune enhancement, and immunization.

4. Investigation of various surface binding macromolecules to determine specificity of binding to particular malignant cell types.

KEYWORDS: Cell surface, Human Ovarian Carcinoma in Cancer, Individualized chemotherapy, Lewis lung carcinoma, Macromomycin, Metastasis, Nude mice, TA3Ha tumor, TA3Kl tumor, Tumor immunogenicity.

Honors and Awards: None

Publications: See current report, Drug Evaluation Branch, Office of the Chief.

SCREENING SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products *in vivo*, and to a limited degree, *in vitro*; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions, and through collaborations with DCT intramural laboratories. Activity against experimental tumors *in vivo* is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. *In vitro* activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for *in vivo* screening, for following fractionation and isolation of active components of crude natural products with correlative *in vivo* activity, for providing information relative to mechanisms of drug actions, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

The Screening Section is currently staffed by five senior professionals and two junior professionals, one on detail from the Office of the Chief, DEB. Principal investigators are: B.J. Abbott (Head); M.C. Baratta (CO); R.I. Geran; N.H. Greenberg, and M.M. Macdonald. Staff are supported by five technical and clerical personnel.

Staff serve as Project Officers for five contracts devoted predominantly to presumptive *in vivo* screening; Battelle Columbus Laboratories (N01-CM-12182), Hazleton Laboratories (N01-CM-23704), Illinois Institute of Technology (N01-CM-81021), Mason Research Institute (N01-CM-12259), and WARF Institute (N01-CM-60494). These contracts and the *in vivo* screening portion of the multifaceted contract, N01-CM-33727 (Arthur D. Little, Inc.) were recompeted during the last year. Thirteen organizations submitted proposals. Seven were selected and we expect these contracts to be operational by the end of FY 1975: Arthur D. Little, Inc. (N01-CM-57006), Battelle Columbus Laboratories (N01-CM-57005), Battelle Frankfurt Laboratories (N01-CM-57008), Hazleton Laboratories (N01-CM-57007), Illinois Institute of Technology (N01-CM-57004), Mason Research Institute (N01-CM-57003), and WARF Institute (N01-CM-53770). Although the number of contractors conducting predominantly *in vivo* screening increased by one, the average test capacity per contract was reduced. Our total *in vivo* screening capacity will be reduced by 8.4% this coming year and an equal amount the following year because of funding limitations. Staff also serve as Project Officers for a contract with Catholic Medical Center (N01-CM-23703) for the evaluation and use of the CD8F1 mouse mammary tumor as a model for human breast carcinoma and for the production of CD8F1 mice for similar studies in other laboratories; and also for two contracts devoted to *in vitro* screening and related new assay development (University of Miami, N01-CM-02225 and University of Wisconsin, N01-CM-33733). In addition, staff, collaborate with the Chief, Drug Evaluation Branch (DEB); Chief, Drug Development Branch (DDB); Chief for Laboratory Research, Drug Research and Development (DR&D); and the Associate Director, DR&D to monitor screening, detailed drug evaluation, and methodology tasks performed under multifaceted contracts with Southern Research Institute (N01-CM-43756), Arthur D. Little, Inc. (N01-CM-33727 and 53765), Bristol Laboratories (N01-CM-90035), Upjohn Co. (N01-CM-43753) Michigan Dept. of Health (N01-CM-33702), Institute Jules Bordet (N01-CM-53840), Japanese Foundation for Cancer Research (N01-CM-22054), Mario Negri Institute (N01-CM-33720), Research Triangle Institute (N01-CM-92019), and Microbiological Associates, Inc. (N01-CM-33728). To assist staff in monitoring the increased data emanating from changes in screening protocols, a Screening Services Contract has been negotiated with Illinois Institute of Technology (N01-CM-43755).

In reviewing biological effects of natural product fractions, the Screening Section establishes directions of effort under additional DR&D contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories as indicated; coordinate total effort with other DR&D and DCT projects; design assays for selection of new active drugs, investigate usefulness and feasibility of new assays; recommend candidate drugs for clinical trial; and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics Area, Clinical Trials Area, and for DCT Decision Network review.

II. Significant Accomplishments

A. Determination of Antitumor Activity of New Agents (Primary Screening)

Primary screening (Stage I) consists of the initial testing of synthetics, plant, fermentation, and animal products submitted to DR&D for antitumor testing. Protocols for screening are designed to uncover, from results of initial testing, a modest number of materials which are then subjected to progressively exacting requirements (Stages II and III) designed to select those with the greatest clinical potential.

Since recent advances in analytical chemistry have made it possible to determine chemical structure and purity from minute quantities of compound, it became increasingly desirable that the initial screening step be accomplished with a minimal amount of compound. In July of 1973 a new presumptive *in vivo* screening protocol was initiated for all new agents. One feature of this "mini-screen" approach was a reduction in the number of mice per test group. During 1974, it became evident that the screening of random synthetic and natural products using 3 mice per dose level - synthetic materials injected on days 2 and 6 against mouse leukemia L1210 and natural products injected days 1-9 against mouse leukemia P388 - was not producing the usual yield of active materials. Consequently, a study was conducted at all appropriate contract laboratories comparing various regimens utilizing 3 and 6 mice per dose level. The results were sent to A. D. Little, Inc. for statistical analysis. Pending completion of this analysis, synthetic materials are being tested either day 1 only; days 1,5, and 9; or days 1-9 (dependent upon the quantity of material). Natural products are tested daily for 9 days. At present, we are using 6 mice per test group. However, the current analysis indicates that the decrease in yield of active materials observed earlier was not caused by the reduction in the number of mice per test group. Screening protocols are now being revised and the "mini-screen" concept will be used for initial screening.

Stage II consists of limited schedule dependency testing designed to exploit test conditions in an effort to increase activity. To pass, a natural product must now demonstaate a higher level of activity (150% T/C) in P388 or it must also be active in L1210. A synthetic must maintain L1210 activity. Stage III is identical for synthetics and natural products, and if the compound possesses a unique chemical structure, consists of testing against secondary tumors (mouse B16 melanoma, mouse Lewis Lung Carcinoma, etc). plus full route and schedule dependency testing. Analogs of compounds already in development are compared with the parent compound in test systems selected on the basis of the biological activity of the latter.

In addition to the initial L1210 and P388 screens, *in vitro* (KB) screening continues for all new crude plant and animal products and fractionation products from crude plant extracts which had demonstrated correlative *in vivo* activity. Synthetic materials received in quantities too small to allow *in vivo* testing are also tested *in vitro*. An effort is made to obtain additional quantities of those demonstrating significant cytotoxicity for further testing *in vivo*.

During calendar year 1974, 39,814 new synthetics, 5,830 new crude plant extractions, 725 new crude fermentation products, and 2,011 new crude animal products were submitted for screening; for a total of 48,380.

B. Selection of Candidate Drugs for Clinical Trial

During 1974, 919 new compounds passed initial screens (Table I). Of these, 218 (24%) demonstrated confirmed activity *in vivo* as follows: 96 against L1210, 118 against P388, and 4 against B16 (Table II). The remaining 701 were active against KB cells in culture and were either inactive *in vivo* or not tested *in vivo* because of limited supply. The DRS reveiwed screening results for 664 compounds. Of the latter, 573 were scheduled for further laboratory investigation, and 91 were reviewed by the DEC. Seven new agents passed DN-2A and proceeded in development toward clinical trial (Table III).

C. Detailed Evaluation of New and Old Drugs of Particular Interest in The Program of the Division of Cancer Treatment

The Screening Section is largely responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN-2A including route and schedule dependency test, combination chemotherapy, studies of comparative effects against fast and slow-growing tumors, and other appropriate studies to determine the extent to which modifications in drug usage might increase each drug's therapeutic efficacy. Such information is reviewed and summarized for Linear Assay Decision Points subsequent to DN-2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups.

The Section maintains the Selected Agents (SAC) file comprising comprehensive records of experimental results for all active drugs and compounds of special interest. At present, the file contains information on 5,536 materials. Approximately one-third of these are being actively pursued on their own merit and the others are included in comparison studies with related drugs. As new data are received, they are reviewed progressively: first by staff; then by the DRS or DEC. When warranted by new experimental findings, these materials are presented to the DN-2A group.

1. Route and Schedule Dependency Studies During 1974, Schedule Dependency studies were initiated for 45 compounds; 27 in L1210, 8 in P388, one in B16, and 9 in a mouse ependymoblastoma system. Follow-up schedule dependency testing was conducted as required. The Screening Committee reviewed 21 compounds and submitted summary reports to the Chief, DEB and for use in clinical brochures. Schedule dependency studies usually involve 3 routes of administration and seven treatment schedules. The principal route is intraperitoneally except for highly insoluble compounds, when the oral route of administration is emphasized.

2. Combination Chemotherapy An offline computer file of animal data for combinations of drugs is maintained. This file includes an index showing which combinations have been tested. We have followed three approaches in planning animal experimentation in this area: the testing of combinations known to be clinically useful

to assess the degree to which enhancement of clinical efficacy is paralleled in animal models; the testing of drug combinations of current clinical interest to determine the optimal conditions for their use with regard to dosage ratio, treatment schedule, and sequence of administration; the testing of untried combinations of clinically active drugs to identify promising combinations prospectively. Experimental studies are both disease and drug oriented. Some pertinent instances in which combined treatment was substantially superior to individual drug treatment are listed in Table IV. Most of the combinations listed are of clinical interest involving the widely used Adriamycin or Cyclophosphamide plus another clinically active drug. However, a number of combinations of alkylating agents including nitrosoureas are listed. The therapeutic synergism of combinations of alkylating agents is particularly noteworthy because one might expect additive toxicity to surpass or equal any additivity of effect against tumor cells. Clearly, this was not the case in these systems since the combination provided an increase in lifespan or cure rate not achieved by individual drug therapy. Moreover, in studies using normal animals, the combined lethality of the alkylating agent combinations listed were shown to be less than additive on the treatment schedules used. The combination chemotherapy studies summarized in Table IV were conducted at Southern Research Institute, Microbiological Associates, Inc., Arthur D. Little, Inc., The Catholic Medical Center, or the Mason Research Institute.

3. Biological Quality Control of Experimental Clinical Formulations We are collaborating with the Clinical Drug Distribution Section, DDB in comparing the *in vivo* activity of formulated drugs with the activity of bulk compound to insure that the antitumor activity has not been impaired or lost during the formulation process. In the past year, comparisons of experimental and/or clinical formulations with bulk material were carried out for 19 drugs in development toward clinical trial.

4. Screening of Structural Congeners of Active Drugs This project calls for (a) the identification by DCT of drug classes of special program interest, in particular the major classes of clinically active drugs; (b) acquisition mainly by contract with DDB, of new structural congeners of clinically active drugs and other rationally designed drugs; and (c) the biological, biochemical, and pharmacological testing of congeners to determine either potential superiority over the parent drug or uniqueness of action. *In vivo* testing of these "analogs" was conducted at Microbiological Associates, Inc. using animal-tumor models selected on the basis of the qualitative and quantitative activity of the parent compound. During 1974 approximately 1,000 new materials were tested in this project. In order to provide rapid feedback to drug synthesis contractors, materials were tested within one week of receipt and results were sent directly to the supplier immediately upon conclusion of each test. Approximately 10% of the compounds tested met the specified activity requirements in at least one

tumor model. However, in most cases, the final decision regarding the recommendation of a structural congener of known clinically active drugs must be based on comparative biochemical, pharmacological or toxicological findings. The main objectives of this project are to identify congeners with experimental activity equal or superior to the parent drug and to provide critical information to aid in the direction of organic synthesis.

D. Methodology (Research and Development)

Concomitant with the search for clinically active materials using current established systems is the search for new animal tumor models that may represent potentially improved assays for primary or secondary screening or for detailed drug evaluation. Some of the more interesting models that are currently under investigation or in actual limited use are described below.

1. The transplantable Ridgway osteogenic sarcoma (ROS) in AKD2F1 mice (Southern Research Institute) is a uniformly fatal solid tumor which is sensitive to many active classes of clinically effective anticancer drugs. The tumor can be staged easily and thus permits a wide variation in experimental design for the simulation of human protocols for individual drug or combination chemotherapy. Established tumors regress under treatment with Actinomycin D (Act. D, NSC 3053), Adriamycin (ADR, NSC 123127), Daunomycin (NSC 82151), Bleomycin (NSC 125066), Cyclophosphamide (CPA, NSC 26271), Melphalan (NSC 8806), 5-Fluorouracil (5-FU, NSC 19893), Cytosine arabinoside (NSC 63878), 6-Mercaptopurine (6-MP, NSC 755), Vincristine (VCR, NSC 67574), and Cis-Pt-II (NSC 119875). It is relatively insensitive to nitrosoureas and a variety of alkylating agents and anti-metabolites excluding those listed above. It is insensitive also to Camptothecin, Na (NSC 100880), Mithramycin (NSC 24559), Vinblastine (VLB, NSC 49842), Prednisone (NSC 10023), and Hexamethylmelamine (NSC 13875). The combinations of CPA plus Act. D, ADR, 6-MP, or 5-FU; and ADR plus Cis-Pt-II were found to be therapeutically synergistic in this system (Table IV.)

2. Under the combined auspices of the Colon Cancer Task Force and DEB, the Southern Research Institute carcinogenically induced and transplanted 82 colon tumors in mice. Four survived the initial transplant and are in serial passage. They show varying degrees of differentiation and at least two are highly metastatic suggesting utility for combined surgical-chemotherapy studies. Various nitrosoureas, 5-Fluorodeoxy-uridine (NSC 27640), Dibromodulcitol (NSC 104800), CPA, I-CPA (NSC 109724), VLB, and ADR showed activity against one or more of these tumors suggesting that they may serve as predictive models for human colo-rectal cancer.

3. The CD8F1 mouse mammary carcinoma developed at the Catholic Med-

ical Center was used for the study of drug combinations and surgical-drug combined treatment of immediate pertinence to the therapy of human breast carcinoma. The tumor was very sensitive to CPA, Melphalan, ADR, and 5-FU; and moderately sensitive to Prednisone. Drug combinations showing potentiating activity in this system are listed in Table IV. The system is eminently suited for combined surgery-drug studies and demonstrated the superiority of surgery followed by Melphalan over surgery alone or Melphalan alone. Of significance was the observation that surgery followed by the triple drug combination of Melphalan, ADR, and 5-FU was substantially more effective than surgery followed by individual drug treatment. The C3H mammary carcinoma used at Southern Research Institute showed biological characteristics and therapy responses similar to the CD8F1 model. However, the incidence of spontaneous tumors in C3H mice has decreased in recent years. This has hampered the further development and use of the C3H model, and it is being discontinued. The CD8F1 model will be used at both the Catholic Medical Center and Southern Research Institute as the mice become available.

4. IITRI is evaluating a renal cell carcinoma that was described in 1973 by Murphy and Hrushesky (JNCI 50, 1013-1025, 1973). The growth characteristics of this tumor - survivors beyond day 60 post tumor implantation - have limited the number of experiments and evaluation parameters that can be studied. Large scale testing with an intrarenal implant appears to be impractical due to the length of time (5-10 minutes) required to implant the tumor. Subcutaneous implants are viable but the tumor ulcerates long before death of the host. Studies are continuing in an attempt to develop a meaningful parameter of response.

5. The group at the Chester Beatty Research Institute has focused on (a) the growth of human tumors in immunologically deprived mice and (b) the utilization of these xenograft systems for detailed evaluation of drugs of clinical interest. Human tumors are grown either in CBA/Lac mice made immunologically incompetent by x-irradiation and thymectomy (T-lymphocyte depletion) or in athymic ("nude") mice. T-cell deprived mice have been used to successfully grow human melanomas and carcinomas of the rectum, ovary, and lung. Attempts to grow human breast carcinomas in deprived mice were unsuccessful. However, within the past year, considerable progress has been made in growing human breast carcinomas in "nude" mice. A sigificant finding during the last year was that the PC6 plasma cell tumor and human lung carcinoma in "nude" mice were sensitive to Hexamethylmelamine (HMM) and are suitable for studies of the mechanism of action of this drug. The human lung carcinoma was not sensitive to CPA. Studies of HMM mechanism have been hampered heretofore because of its general inactivity against mouse tumors. Current results suggest that HMM *per se* has no alkylating activity but that it may act *in vivo* after conversion to alkylating methoylol derivatives. However,

the active metabolites of HMM appear to act differently from the active metabolite of CPA.

III. Related Projects

For proper function of the screening program, all contract laboratories must employ tumor lines of uniform biological characteristics and host animals of uniform high quality. To achieve this, the Screening Section maintains frozen tumor banks at specified laboratories and from these, new tumor lines are dispensed to all screening laboratories at specified intervals. These tumor lines are monitored by the Screening Section and if it appears that the characteristics of a tumor line in use may be changing, new tumor lines are requested and sent to all contract laboratories from the tumor bank.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section, DR&D. Selected contract laboratories perform studies related to the diagnosis and control of animal disease and to provide the controls necessary for maintenance of the quality of the animals utilized in the program.

The Screening Section, as the major user of the DR&D data processing system, continues to work closely with AIS by advising and assisting in the planning of modifications and the monitoring and review of data necessary to an ongoing data processing system. In addition, we continue to review for need, all requests for special queries to our data files.

IV. Proposed Course of Projects

The Screening Section will continue the supervision of testing and the evaluation of data for synthetic compounds, plant, animal and fermentation products which are submitted to the DR&D. This includes the continuous evaluation of primary screening, the logistics, criteria, and tumor test systems. Basic research and methodology will be carried out under contract for related projects of interest to DCT, NCI. This research is aimed primarily at devising new and improved antitumor assays, the evaluation of new systems for their potential use as screening tools, and the methodology required prior to establishing their use in several contract laboratories.

Publications

See Current Report, Drug Evaluation Branch, Office of the Chief.

TABLE I

STATUS OF ACTIVE COMPOUNDS

CALENDAR YEAR 1974

New Actives	919
No. of compounds reviewed by	
DRS	664
DEC	91
Total Reviewed	755
Passed DN2	7
Route and Schedule Dependency Experiments Scheduled	45
No. of compounds reviewed for schedule dependency by Screening Committee	21

TABLE II

NEW ADDITIONS TO THE SELECTED AGENTS LISTING

CALENDAR YEAR 1974

Total Number of Materials	Total number for each Tumor System	
218	4	B16
	118	P388
	96	L1210
701	701	KB(cell culture)

TABLE III

COMPOUNDS ASSIGNED TO DN-2 (OR ABOVE) FOR THE FIRST TIME IN 1974

NSC NO.	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT
143095	Pyrazole-3-carboxamide, 4-hydroxy-5-β-D-ribofuranosyl- Pyrazomycin	November 11	Clinical Trial
157365	Neocarzinostatin, crystalline antibiotic	January 28	Japanese Clinical Trial
163501	5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-	November 11	L-1210
170105	Macromomycin	January 28	B16
172755	Glycine, N-[1-oxo-5-(1H-purine-6-ylthio)pentyl]-, ethyl ester Butocin	May 20	Czechoslovakian Clinical Trial
176319	Quinolinium, 6-amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]-phenyl]amino]carbonyl]phenyl]amino]-, dibromide	January 28	Replaced NSC-113089 L1210 and P388
178248	D-Glucopyranose, 2-[[[(2-chloroethyl)-nitrosoamino]carbonyl]amino]-2-deoxy- Chlorozotocin	May 20	L1210

TABLE IV

SOME THERAPEUTICALLY POTENTIATING DRUG COMBINATIONS OF INTEREST

Drug (NSC Numbers)	Test System*
Adriamycin (123127, ADR) Plus	
Vincristine (67574)	PS
Cytosine arabinoside (63878, Ara-C)	PS
Methotrexate (740, MTX)	PS
Melphalan (8806)	C3, CD
Cyclophosphamide (26271, CPA)	LE, PS, C3, CD, ROS
DTIC (45388)	LE, C3, LL, B1
CCNU (79037)	LE
MeCCNU (95441)	B1, C3, PS
Cis-Pt-II (119875)	PS, ROS
ICRF-159 (129943)	LE
5-Fluorouracil (19893, 5-FU)	PS, CD
ADR + CPA + 5-FU	CD
ADR + Melphalan + 5-FU	CD
Cyclophosphamide (26271, CPA) Plus	
BCNU (409962)	AK, LE, P4
MeCCNU	AK, SA, LL, CA, C3, B1
Ara-C	AK, LE
6-Mercaptopurine (755)	CA, ROS
Actinomycin D (3053)	ROS
5-FU	ROS, CD
5-Fluorodeoxyuridine (27640, FUdR)	C3
Melphalan	LE, ROS
BCNU or CCNU + Ara-C	LE
BCNU + FUdR	C3
Melphalan + MTX + 5-FU	Rat Ma Ca 13762

* AK - AKR lymphoma, B1 - B16 melanoma, CA - Carcinoma 755, CD - Mammary carcinoma of CD8F1 mice, C3 - Mammary carcinoma of C3H mice, LE - leukemia L1210, LL - Lewis Lung carcinoma, PS - Leukemia P388, P4-Leukemia P-1534, ROS - Ridgway osteogenic sarcoma.

ANNUAL REPORT

LABORATORY OF EXPERIMENTAL CHEMOTHERAPY

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The laboratory conducts fundamental research in experimental chemotherapy through specific programs in tumor biology and immunochemotherapy. These programs include in tumor biology such areas as the investigation of the molecular basis of drug specificity, individualization of therapy and clinical correlations, biology of cell surface in relation to neoplastic transformation, reversibility and therapy, the fundamental basis and therapy of invasiveness and metastasis, models for multiple combination chemotherapy based on biological and biochemical parameters of drug action, and studies of adjuvant chemotherapy. The program in biochemistry includes studies on the biochemical characterization of the cell cycle, the biochemistry of cell surface, alteration of active sites and biochemistry of adhesiveness, the development of biochemical systems for identification and study of new agents and investigations on the biochemistry of drug action. The immunochemotherapy program includes investigation and development of screening and evaluation systems for immune suppression and immune enhancement, investigation of therapy-related immunologic phenomena and selected immunochemotherapy studies in drug chimerism, adoptive immunity plus chemotherapy, humoral antibody plus chemotherapy, antigenic alteration plus chemotherapy, immunogenetics and chemotherapy and immunochemotherapy of viral-induced tumors.

The Laboratory of Experimental Chemotherapy currently includes the Office of the Chief (A. Goldin) and two sections: Tumor Biology (R. K. Johnson) and Immunochemotherapy (E. Bonmassar and D. Houchens).

Two Individual Project Reports are included this year for the Laboratory of Experimental Chemotherapy:

(a) Tumor Biology and Chemotherapy, Project No. Z01 CM 07100-02

(b) Immunochemotherapy Studies in Various Tumor Systems, Project No. Z01 CM 03577-02

During this year of operation of the Laboratory of Experimental Chemotherapy, the two activated sections, Tumor Biology and Immunochemotherapy, have made good progress in findings on special problems.

Project No. Z01 CM 03575-02
1. Office of Associate Director For Drug Research and Development, Division of Cancer Treatment, NCI
2. Laboratory of Experimental Chemo.
3. NCI-Building 37, Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1974 through June 30, 1975

Project Title: Chemotherapy and Combined Modality Investigations of Antitumor Agents

Previous Serial Number: NCI 3575

Principal Investigator: Abraham Goldin

Co-Principal Investigators: R. K. Johnson, E. Bonmassar, D. P. Houchens, A. W. Schrecker, J. M. Venditti, O. C. Yoder

Cooperating Units: G. Cudkowicz, State University of New York at Buffalo; J. M. Venditti and Staff, Drug Evaluation Branch, DCT, NCI; H. B. Wood, Jr., R. R. Engle, J. Driscoll and J. Douros, Drug Development Branch, DCT, NCI; J.A.R. Mead, Experimental Therapeutics, DCT, NCI; R. Gallo, Human Tumor Cell Biology Branch, DCT, NCI; S. K. Carter, Acting Deputy Director, DCT, NCI; M. Slavik, Cancer Therapy Evaluation, DCT, NCI; H. E. Skipper, F. M. Schabel, Jr. and Staff, Southern Research Institute; I. Kline, S. Vadlamudi and M. Kende, Microbiological Associates, Inc.; I. Wodinsky, A. D. Little; E. Frei, III, Children's Cancer Research Foundation, Boston; F. M. Huennekens, Scripps Clinic and Research Foundation, La Jolla; A. DiMarco, Farmitalia, Milan, Italy; A. Nicolin, University of Milan, Italy; S. Garattini, Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

Man Years

Total: 5
Professional: 4
Others: 1

Project Description:

This project in the Office of the Associate Director, Drug Research and Development encompasses the following objectives:

I. To contribute to the planning and coordination of the chemotherapy and combined modality research program of Drug Research and Development and application to drug development and total program.

II. To conduct fundamental investigations on special problems of program interest.

III. To interdigitate with the Chemotherapy Laboratory, Drug Evaluation and Drug Development programs of Drug Research and Development and other preclinical programs in the Division of Cancer Treatment, in structure-activity studies and in the exploration of new and potentially useful biological and biochemical methodology for evaluation and improvement of drug therapy and in special problems and new approaches.

IV. To collaborate with the clinical program, Division of Cancer Treatment in areas interrelating preclinical and clinical investigations.

V. Conduct of international activities including maintenance of chemotherapy offices in Europe and Asia, arrangements for acquisition of test materials, screening of new agents abroad and surveillance for and acquisition of new antitumor agents.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

International Activities

There has been information exchange of most recent developments in chemotherapy, procurement of synthetic agents and natural products for screening and identification of new compounds of potential interest for chemotherapy.

Close liaison is being maintained with investigators in leading institutions including universities, medical schools and pharmaceutical houses abroad. There is active contact with the Chemotherapy Committee of the International Union Contra Cancrum, the European Organization for Research and Treatment of Cancer (EORTC), the World Health Organization in Geneva and the International Agency for Research in Cancer of the World Health Organization. The Division of Cancer Treatment Chemotherapy Offices at the Institut Jules Bordet, Brussels, Belgium and at the Cancer Institute, Tokyo, Japan are making progress in providing a means for close collaboration with the preclinical and clinical cancer chemotherapy programs of Europe and Asia. The chemotherapy offices are functioning successfully in the gathering and exchange of scientific knowledge for the further development of cancer chemotherapy. They are significantly increasing the input of new drugs and screening data into the chemotherapy screening program of the National Cancer Institute and stimulating collaboration in the study of new anticancer drugs. The chemotherapy offices are providing a means for the continuous review, abstracting, and where necessary, translating of significant foreign reports.

Dr. Omar Yoder, serving as an NCI Division of Cancer Treatment officer at the European office, has continued to make excellent progress in furthering the overall collaboration in Europe. He has expanded and maintained contacts with pharmaceutical houses, chemical industry and universities in the acquisition of new compounds for screening and in the exchange of preclinical and clinical information. In addition to his activities at the Jules Bordet Institut he has been making visits to important institutions in the various countries of Europe, working in collaboration with the European Organization for Research and Treatment of Cancer (EORTC) as well as the other European organizations. This has resulted not only in enlarging the contacts for materials for screening but also in providing extensive information on drugs and modalities of therapy of clinical interest. The Jules Bordet Institut is serving as the data and coordinating center for the E.O.R.T.C. which gives our office an excellent tie-in with all of the preclinical and clinical chemotherapy activities in Western Europe. The screening program at the Jules Bordet Institut has exceeded the projected figures for the screening of new antitumor agents. The materials tested in the screening program are collected by the DCT office in Europe. A screening program is also functioning successfully at the Mario Negri Institute in Milan for the testing of compounds collected primarily in Southern Europe. A chemotherapy program has been instituted at the Chester Beatty Institute in England and assistance was provided to a screening program at the Cancer Institute in Dublin, Ireland. A program on plant collection, extraction and screening at the Weizmann Institute in Rehovoth, Israel has uncovered several materials of interest. The screening program at the Cancer Institute in Tokyo as part of the "Anticancer Drug Evaluation Center" has made good progress in the testing of candidate agents. The materials that are being tested in the screens are being collected primarily from pharmaceutical houses and universities in Japan. The data collected in the screening programs in both Europe and Asia are entered into the Drug Research and Development computer program. The DCT offices in both Brussels and Tokyo continue to publicize extensively the DCT program in the various countries of Europe and Asia.

As part of the U.S.-U.S.S.R. Cooperative Medical Agreement a combined clinical and preclinical team visited the Soviet Union for exchange of information and for the expediting of the overall collaborative effort. A collaborative program has been in progress for the interchange and evaluation of Soviet and American drugs. This has been functioning well and some manuscripts involving joint publication are in progress.

Visits were made by A. Goldin as follows:

1. A visit was conducted, accompanied by Drs. Seymour Perry and Omar Yoder to Dr. K. H. Schmidt-Ruppin at Ciba-Geigy, Basel, Switzerland. Ciba-Geigy has now made a serious commitment to the area of cancer chemotherapy and the visit was prompted by an expressed interest of Dr. K. H. Schmidt-Ruppin in the possibility of further collaboration with our institute in the area of cancer chemotherapy. Such collaborative arrangements were made and it was agreed to supply our program with specific compounds of interest.

Following the visit to Basel, we attended a meeting of the EORTC Screening Committee in Brussels at which antitumor drugs at various stages of development for the clinic were discussed. The Screening Committee is a relatively new arm of the EORTC program.

2. A visit was made to the Soviet Union to participate in a Joint U.S.-U.S.S.R. Meeting on Lung Cancer and a Joint U.S.-U.S.S.R. Chemotherapy Review. In the Chemotherapy Review Meeting the clinical experience with CCNU, DTIC and hexamethylmelamine from the United States and asaley from the Soviet Union was reviewed. Laboratory and clinical data were presented on the nitrosourea derivatives being studied in the United States and data on new antitumor drugs were presented by the Institute of Chemical Physics of the U.S.S.R. Academy of Science. In addition, preclinical and clinical investigations were presented by them on nitrosourea. The preclinical data on various drugs including asaley, olivomycin, palphicerin, phenesterol, distron, fluorodopan, hanerol, colkhisin, aton, carminomycin and ftorafur were reviewed, both by the Soviet and American scientists. Studies with ftorafur in comparison with 5-fluorouracil were conducted at NCI as a joint program of the American and Soviet scientists and the data was presented.

3. Participation and presentation of paper at Workshop on New Animal Models for Chemotherapy of Human Solid Tumors, in Budapest, Hungary. The workshop was organized by the International Union Against Cancer and the local sponsor was the Hungarian National Institute of Oncology. The primary objective of the Workshop was to discuss new tumor models that might be useful in identifying new antitumor agents and more effective treatments against human solid tumors. Basic questions concerning human tumor heterotransplantation models were discussed during the workshop. In addition to the standard models currently being employed in screening, the range of models discussed included the following: tumor models utilizing perfusion; a "sandwich model" in which the tumor cells are grown in a thin sheet created by skin flaps; diffusion chamber models; human tumor grown in the hamster cheek pouch; the B cell model (B mouse) for which the thymus is removed, the host lethally irradiated, and then "rescued" by the administration of B cells; human tumor xenografts under the kidney capsule; tumor growth in privileged sites; and tumor growth in the

athymic mouse. It was clear that there is great interest in heterotransplantation studies utilizing athymic mice.

4. Participation and presentation of papers at a symposium on Clinical Pharmacology with Special Reference to Cancer and the Second International Symposium on Adriamycin. These symposia were conducted at the Jules Bordet Institut in Brussels under the auspices of the European Organization for Research on Treatment of Cancer (EORTC).

Participation and presentation of paper at an Adriamycin symposium in Frankfurt, Germany, with Dr. D. Schmahl of the German Cancer Society presiding. There was a specific interest in the adriamycin symposium in Frankfurt since there is a desire to increase the interest in chemotherapeutic and related modalities of therapy in Germany. Also, Deutsche Farmitalia has introduced adriamycin (Adriblastin®) in Germany.

Visited and presented a seminar at the Oncological Institute in Bucharest, Romania which is under the direction of Professor O. Costachel. During the visit there was the opportunity to meet with the various groups in the institute and to conduct individual discussions with the investigators in both preclinical and clinical areas.

Visited and gave a talk at the Oncologic Institute in Sofia. The department of experimental therapy under Dr. J. Stoichkov is a young, vigorous and developing group.

Participation and presentation of papers at the Second National Congress of Oncology with International Participation in Varna-Druzhba, Bulgaria. This congress was conducted under the auspices of the Ministry of Public Health, Medical Academy-Center of Oncology, Bulgarian Oncological Society. Although most of the participants were from Bulgaria, there were a large number of countries represented.

5. Conducted a site visit to the Weizmann Institute of Science in connection with two PL-480 agreements, agreement 06-124-1 and 06-025-1. The former agreement (06-124-1) is with Dr. D. Lavie and staff and pertains to plant collection in Israel, extraction and purification and in vivo antitumor testing. This agreement was scheduled to terminate March 1, 1975 and Dr. Lavie is seeking additional funding for its continuance. Dr. Lavie has built up a smoothly functioning program under the PL-480 agreement and it is considered that its further funding would be important. He has now received funding for continuation of a portion of the program from the United States-Israel Binational Science Foundation.

At the Weizmann Institute additional visits were made to the laboratories of Dr. Michael Feldman, Nathan Trainin, Isaac Berenblum, Michael Sela and others for discussion of ongoing programs in immunology and other areas as related to our chemotherapy program.

PL-480 agreement 06-025-1 has terminated and the purpose of the site visit on this agreement was to review the overall accomplishments on the program and the final status of programs in progress. The agreement pertains to the study of pharmacological properties of synthetic oligo and macro-molecules, and discussions were conducted with Dr. Ephraim Katchalski and Dr. Abraham Novogrodsky concerning the further application of the findings in their investigations with the synthetic oligo and macro-molecules to our program.

Presented a paper at the XVth International Congress of the Society of Hematology in Jerusalem.

With Drs. S. Carter, S. Schepartz, J. Venditti and O. Yoder contributed to the organization and participated in an International Conference on Screening Methodology for Antitumor Drugs held in Geneva under the overall auspices of WHO. This program was organized in order to further world-wide cooperation in obtaining a cure for cancer. The international conference was attended by leading chemotherapists of most of the countries conducting chemotherapy programs. The primary objectives to be attained included: (1) compilation and publication of test systems utilized in cancer chemotherapy screening; (2) standardization of test systems; (3) discussion of status and establishment of animal genetic centers and their distribution; (4) the listing and establishment of tumor cell banks; (5) discussion of the possible establishment of a centralized data bank; (6) exchange of active drugs; (7) exchange of publications; (8) collaborative publication; (9) establishment of a standard reference screen; (10) the establishment of an international tumor spectrum for drug evaluation; (11) research on new model systems; (12) discussion of future plans for cooperation and collaboration. Based on the presentations and discussions at the conference a document was prepared and received unanimous approval entitled, "General Agreement by Participants on World-Wide Cooperation On Antitumor Drug Screening and Drug Evaluation."

6. Attendance and participation at the XIth International Cancer Congress, Florence, Italy. Presented a paper at the conference "Advanced in Cancer Chemotherapy: Combination Versus Single Drug Chemotherapy." Five additional papers were presented with collaborating investigators.

7. A. Goldin and S. Carter attended and presented papers at the first formal meeting of Scientific Exchange in the Chemotherapy of Cancer under the United States-Japan agreement and conducted a site visit to the Tokyo Liaison Office. The meetings were successful and a definite step in the direction of collaboration with the Japanese at both the preclinical and clinical levels in cancer investigations.

The following talks describing various aspects and results of our program were given abroad during the year by A. Goldin and colleagues:

1. Present Status of Tumor Models; Usefulness and Limitations of Murine Models. Presented at a "Workshop on New Animal Models for Chemotherapy of Human Solid Tumors" Budapest, Hungary.

2. Predictability of Animal Test Systems for Clinical Cancer Chemotherapy. Presented at a "Symposium on Clinical Pharmacology with Special Reference to Cancer" Jules Bordet Institut, Brussels, Belgium.

3. Antitumor Effects of Adriamycin in Comparison with Related Drug and in Combination Chemotherapy. Presented with R. K. Johnson as co-author, at the "Second International Symposium on Adriamycin" Jules Bordet Institut, Brussels, Belgium.

4. Adriamycin Activity in Experimental Tumor. Presented at Adriamycin Symposium in Frankfurt, Germany, with R. K. Johnson as co-author.

5. Concepts in Screening and Evaluation for Cancer Chemotherapy. Presented at the Oncological Institute in Bucharest, Romania.

6. The Drug Research and Evaluation Program at the National Cancer Institute. Presented at the Oncologic Institute in Sofia, Bulgaria.

7. Preclinical Investigations of Antitumor Agents in Relation to the Clinic. Presented at the "Second National Congress of Oncology with International Participation" in Varna-Druzhba, Bulgaria.

8. Experimental Investigations in Combination Chemotherapy. Presented at the "Second National Congress of Oncology with International Participation" in Varna-Druzhba, Bulgaria.

9. Preclinical Approaches to Drug Selection of Antileukemic Agents. Presented at "XVth International Congress of the Society of Hematology" in Jerusalem, Israel.

10. Experimental Grounds for Combination Chemotherapy. R. K. Johnson as co-author. Presented at "XIth International Cancer Congress" in Florence, Italy.

11. Immunologic Cross-Reactivity of Two Leukemic Sublines. (A. Nicolin, A. Bini, F. Franco and A. Goldin). Presented at "XIth International Cancer Congress" in Florence, Italy.

12. Allograft Reactivity of Mice Against a Radiation-Induced Lymphoma Incompatible for H-2K-Ir of H-2 Complex. (E. Bonmassar, A. Bonmassar, M. Fioretti and A. Goldin). Presented at "XIth International Cancer Congress" in Florence, Italy.

13. The Uptake of 5-Iododeoxyuridine as a Measure of Tumor Growth In Vivo. (M. Fioretti, E. Bonmassar, D. Houchens and A. Goldin). Presented at "XIth International Cancer Congress" in Florence, Italy.

14. Combination Treatment with Vinblastine (VBL, NSC 49842) and 5-Azacytidine (5-Aza C, NSC 102816) in Mice Bearing Leukemia L1210. (S. Vadlamudi, A. Miyakawa, W. Thomas, R. K. Johnson and A. Goldin). Presented at "XIth International Cancer Congress" in Florence, Italy.

15. Effects of Adriamycin in Nucleic Acid Biosynthesis by L1210 and P388 Leukemia Cells In Vivo. (R. K. Johnson, B. T. Garibjanian and A. Goldin). Presented at "XIth International Cancer Congress" in Florence, Italy.

16. Cytosine Arabinoside and Cyclocytidine as Examples of the Problems of Schedules Dependency Testing. Presented at the symposium "Comparative Study on Cytosine Arabinoside and Cyclocytidine, Experimental and Clinical" in Tokyo, Japan.

17. Combination Experimental Studies with Cytosine Arabinoside and Cyclocytidine. Presented at the symposium "Comparative Study on Cytosine Arabinoside and Cyclocytidine, Experimental and Clinical" in Tokyo, Japan.

18. The Potential Impact of Experimental Studies on the Clinic. Presented at the U.S.-Japan Joint Workshop, Tokyo, Japan.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

Structure-activity studies have been continued on the antitumor activity of adriamycin and related drugs. Structural alteration have involved the aglycone and the sugar moieties. Although some of the analogues of adriamycin such as the 14-octanoate derivative appeared to be about as effective as adriamycin in the treatment of leukemia L1210, none of them, as yet, have proven to be more effective.

In a comparison of adriamycin, daunomycin, rubidazone and carminomycin in the treatment of leukemia L1210, adriamycin was most effective. Rubidazone was somewhat more effective than daunomycin. Daunomycin and carcminomycin were about equally effective. Adriamycin was also more effective than daunomycin, rubidazone or carminomycin in the treatment of leukemia P388. Structure-activity studies are being continued with Drs. Johnson, Wood, Engle, Driscoll, Mead and Venditti on congeners of known antitumor agents.

II. Mechanism of Action of Antitumor Agents

A. Methotrexate Transport. (A. W. Schrecker and F. M. Huennekens)

Suggestion that enhanced transport of metabolites into malignant cells is related to decreased intracellular levels of cyclic AMP has prompted a study of the effects of exogenous cyclic nucleotides and inhibitors of phosphodiesterase on uptake of methotrexate (MTX), which is known to enter mammalian cells by the 5-methyltetrahydrofolate (5-$MeFH_4$) transport system. Initial rates of MTX uptake by leukemia L1210 cells were inhibited by cyclic AMP, cyclic GMP and their dibutyryl derivatives, as well as by 1-methyl-3-isobutylxanthine, a potent cyclic AMP phosphodiesterase inhibitor. Cyclic nucleotides also depressed uptake of 5-$MeFH_4$ and suppressed proliferation of L1210 cells in culture. The results are consistent with pleiotypic control of transport in L1210 cells and may suggest that relatively lower levels of cyclic AMP in some leukemic cells lead to an increased rate of MTX uptake and contribute to the selective cytotoxic effect of the drug.

B. Characterization of DNA Polymerases. (A. W. Schrecker and R. C. Gallo)

In a program which is continuing, template and primer characteristics of normal cellular DNA polymerases present in human lymphocytes and of the viral reverse transcriptase have been under investigation. By use of synthetic polynucleotide templates and oligonucleotide primers it is hoped to characterize and distinguish more definitively the three main cellular polymerases (α, β, γ) and the reverse transcriptase. Preliminary findings show that the template-primer combinations polydeoxythymidylate-oligoadenylate and polyadenylate-oligouridylate are used in a preferential manner by DNA polymerase γ, an enzyme not readily distinguished from reverse transcriptase.

III. Immunochemotherapy

A. Adoptive Immunotherapy in BALB/c X DBA Mice Bearing an Immunogenic Subline of L1210 Leukemia. (A. Nicolin, G. Canti and A. Goldin)

That treatment with 5,(3,3-dimethyl-triazeno) imidazole-4-carboxamide (DIC) *in vivo* may have induced new antigen(s) on L1210/Ha leukemia cells is supported by the finding of specific immune spleen cells in sensitized mice to the DIC-treated L1210/Ha leukemia (L1210/Ha/DIC). An infusion of immune spleen cells "cured" immunosuppressed CDF_1 mice bearing the L1210/Ha/DIC leukemia and did not exhibit any therapeutic activity in mice bearing 10^6 cells of the parental L1210/Ha leukemia. Spleen cells immune to syngeneic or allogeneic tumor cells did not protect the mice challenged with 10^6 L1210/Ha/DIC cells.

B. Allograft Reactivity of Mice Against a Radiation-Induced Lymphoma Incompatible for the H-2K-Ir Regions of H2. (A. Bonmassar, M. Fioretti, E. Bonmassar and A. Goldin)

Further studies were conducted on the dilution escape phenomenon. B10.A(5R) mice reject 10^7 cells of a radiation-induced lymphoma of B10.A origin (LAF-17) incompatible for the H-2K-Ir regions of the H-2 complex. However, most of the mice of B10.A(5R) strain succumb after a challenge of 10^4 cells of the same lymphoma. The results

> indicate that the mice challenged with 10^7 lymphoma cells showed a strong allograft reaction, whereas the allograft reaction of the animals injected with 10^4 cells was weak or absent, in spite of a rapid increase of antigen availability due to allogeneic tumor growth. In addition, it was observed that another radiation-induced lymphoma of B10.129(5M) origin was immunosuppressant for allogeneic mice and prevented their allograft response to high inocula of LAF-17 cells. It was concluded that the challenge of incompatible mice with a limited number of LAF-17 tumor cells did not provide initially an adequate antigenic stimulus and immunosuppressed the recipients. When the transplanted tumor reached the critical size necessary to promote an efficient allograft response, the host was incapable of rejecting the tumor and succumbed with generalized lymphoma.

The remainder of the major findings in collaborative program are summarized in the annual reports of the Tumor Biology and Immunochemotherapy Sections of the Laboratory of Experimental Chemotherapy and in the annual reports of the Drug Research and Development Branches.

Proposed Course of the Project

The various aspects of the project will be continued and modified in accordance with laboratory findings and clinical interest.

Continued emphasis will be placed on developing the international program and world surveillance for drugs for screening, drugs of interest for the clinic and new modalities of therapy. Continued emphasis will be placed on activities related to the planning and coordination of the total DR&D research program and on the further development of the preclinical-clinical interrelationships with respect to the predictability value of animal test models, combination chemotherapy and combination modalities. It is planned to develop further the Laboratory of Experimental Chemotherapy and in the extension of its activities to involve it in collaborative investigations with the other segments of Drug Research and Development and the Division of Cancer Treatment as well as in collaborative research in the United States and abroad.

Honors and Awards:

A. Goldin received the H.E.W. Superior Service Award.

Keyword Descriptors:

Cancer chemotherapy, combined modalities, special investigations, research coordination and collaboration, international activities.

Publications:

1. Bonmassar, A., Fioretti, M. C., Bonmassar, E., and Goldin, A.: Allograft reactivity of mice against a radiation-induced lymphoma incompatible for the H-2K-Ir regions of H2. Europ J. Cancer. In press.

2. Bonmassar, E., Menconi, E., Goldin, A., and Cudkowicz, G.: Escape of small numbers of allogeneic lymphoma cells from immune surveillance. J. Nat. Cancer Int. Pt. 2, 53: 475-479, 1974.

3. Goldin, A., and Johnson, R. K.: Adriamycin activity in experimental tumors. In: Ghione, M., Fetzer, J., and Maier, H. (Eds.): Ergebnisse der Adriamycin-Therapie. Berlin, Springer-Verlag, 1975, pp. 3-13.

4. Goldin, A., and Johnson, R. K.: Antitumor effects of adriamycin in comparison with related drugs and in combination chemotherapy. Presented at "IInd International Symposium on Adriamycin" Institut Jules Bordet, Brussels, Belgium, May 13-18, 1974.

5. Goldin, A., and Johnson, R. K.: Experimental antitumor activity of adriamycin. Cancer Chemother. Rep. In press.

6. Goldin, A., and Johnson, R. K.: Experimental grounds for combination chemotherapy. In Proceedings, Eleventh International Cancer Congress, Florence, Italy. In press.

7. Li, C. P., Goldin, A., and Hartwell, J. L.: Antineoplastic substances from the sea: a review. Cancer Chemother. Rep. Pt. 2, Vol. 4, No. 3, 97-129, 1974.

8. Nicolin, A., Bini, A., Franco, P., and Goldin, A.: Cell-mediated response to a mouse leukemic subline antigenically altered following drug treatment in vivo. Cancer Chemother. Rep. Pt. 1, 58: 325-330, No. 3, 1974.

9. Nicolin, A., Canti, G., and Goldin, A.: Adoptive immunotherapy in BALB/c X DBA/2 Cr F_1 mice bearing an immunogenic subline of L1210 leukemia. Cancer Res. 34: 3044-3048, 1974.

10. Venditti, J. M., and Goldin, A.: Therapeutic synergism in animal models-increased antitumor specificity resulting from diminished toxicity for the host. Presented at Symposium on Clinical Pharmacology, Jacksonville, Florida, March 12-13, 1973. Biochem. Pharmacology, Supplement No. 2, 1974, pp. 97-129.

Project No. Z01 CM 03577-02
1. Laboratory of Experimental Chemotherapy
2. Immunochemotherapy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1974 through June 30, 1975

Project Title: Immunochemotherapy Studies in Various Tumor Systems

Previous Serial Number: NCI 3577, Part of NCI 3575

Principal Investigator: Enzo Bonmassar

Co-Principal Investigators: David Houchens and Abraham Goldin

Other Investigators: Franca Campanile, Lucio Crino, Randall K. Johnson, Artemio Ovejera

Cooperating Units: L. Cahan and A. Daniel, Surgical Neurology Branch, NINDS; M. Kende, Microbiological Associates, Inc., Bethesda, Maryland; M. Fioretti, Institute of Pharmacology, School of Medicine, University of Perugia, Perugia, Italy; B. Giovanella, Stehlin Cancer Foundation, Houston, Texas

Man Years

Total: 5.5
Professional: 4
Other: 1.5
Plus animal care support from Mammalian Genetics and Animal Production Section, DR&D, DCT, NCI

Project Description:

Objectives:

I. To study the antigenic changes occurring in tumors following treatment with antineoplastic agents in vivo.

II. To monitor the growth of neoplastic cells in tumor bearing mice in order to determine and improve the efficacy of combined chemoimmunotherapy.

III. To develop a program employing athymic (nude) mice for the growth and treatment of human tumors.

IV. To develop appropriate models for investigation of interactions between antitumor agents and the host's immune response and the application of immunotherapy.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Studies on Antigenic Changes of Tumor Cells Induced by Drug In Vivo. (E. Bonmassar, D. Houchens, M. Kende and A. Goldin)

The antigenic alterations that can be induced in tumor cells by DIC were utilized in immunochemotherapy against challenge with the parental tumor line. Mice were challenged with LSTRA lymphoma. One day later the DIC transformed LSTRA was administered, followed by graded doses of BCNU two days later. At the 10^3 challenge level there were 7 survivors out of 8 animals challenged when 18 mg/kg of BCNU was used. When only BCNU was used, without DIC transformed cells, there were no survivors. ^{51}Cr release cellular cytotoxicity assays to determine if there are different antigens present in the DIC transformed cells were performed. Mice immunized with DIC lines were used as spleen cell donors. Both parental and DIC lines were able to inhibit cytotoxicity of DIC-transformed target cells although the DIC line produced slightly more inhibition indicating that the antigens were the same although more strongly expressed.

II. Monitoring Tumor Cell Growth and Tumor Inhibition. (E. Bonmassar, D. Houchens, M. Fioretti, M. Kende and A. Goldin)

The method described in last year's annual report to monitor DNA synthesis associated with tumor cell growth was further developed and employed. DNA synthesis of tumor cells in spleen, lung and liver of mice bearing spontaneous or transplanted lymphomas or Lewis lung carcinoma was determined by measurement of incorporation of ^{125}IUdR. Uptake values of blood from leukemic AKR mice were significantly higher than non-leukemic controls. There was a direct relationship between isotope uptake values and the extent of neoplastic growth. The ^{125}IUdR uptake was also found to be applicable to determination of inhibition of tumor growth in drug-treated animals.

III. Nude Mouse Studies

A. Drug Induced Immunogenic Changes in Lymphoma Lines in Nude Mice. (F. Campanile, D. Houchens, E. Bonmassar and A. Goldin)

Highly immunogenic sublines of L1210 and LSTRA leukemias were obtained in athymic mice after treatment with DIC *in vivo*. Conventional mice that were syngeneic for the parent tumor, rejected the DIC-treated sublines while the nude mice did not reject them. This indicated that the transplantation resistance to these tumors is T-cell dependent.

B. Human Tumor Growth in Nude Mice. (D. Houchens, F. Campanile, L. Crino, B. Giovanella and A. Goldin)

A program to grow human tumors in athymic mice for chemotherapy and immunotherapy studies has been developed. A new laboratory with special air filtration systems is being built for these studies. At the present, nude mice on an NIH Swiss background are being used. They have been injected with human lung tumor and ALL lymphoblasts from tissue culture as well as fresh human fibrosarcoma and liposarcoma tissue from nude mice supplied by Dr. Giovanella. Toxicity studies have been carried out in nude mice with 10 chemotherapeutic agents. The results indicate that the mice can tolerate the drugs as well as normal strains.

IV. Immunotherapy and Immunochemotherapy

A. Non-specific Stimulation of Immune System. (D. Houchens and R. K. Johnson)

Levamisole and tetramisole were shown to be ineffective in several murine tumors but to have variable activity in a primary Moloney sarcoma virus-induced rhabdomyosarcoma. Neither drug had any effect in mice immunosuppressed with cyclophosphamide as previously reported with BCG from this laboratory.

Studies were begun with the clinical preparation of *Corynebacterium parvum* in various tumor systems. The preparation had no activity against L1210 but produced significant increases in ILS when it was tested against P388. Immunochemotherapy tests employing adriamycin and *C. parvum* against Lewis lung and P388 showed synergistic effects with the combination treatment. Current tests indicate that the effect of C. parvum can be demonstrated if the agent is given before P388 as well as after.

B. Immunotherapy Studies in a Murine Glioma Model. (D. Houchens, L. Cahan and A. Daniel)

The intracerebral glioma tumor GL-26 which was used in developing the immunochemotherapy model reported on last year was continued to be used to determine other immunological parameters affecting this system. It was determined that neuraminidase treatment is not necessary in treatment of the cells that are used for immunization. Mitomycin treatment alone is sufficient. Various adoptive immunotherapy procedures were used. There is some indication that spleen cells from immune animals are effective in transfer of immunity if given before tumor challenge. On the other hand, lymph node cells do not confer immunity on the mice. Various extractions have been performed on the tumor cells to isolate the active antigenic portion. These preparations have been tested but no determination has yet been made as to the portion containing the immunogen.

Significance to Biomedical Research and the Program of the Institute:

The studies are part of a program to set up new strategies for the immunochemotherapy of malignant tumors. Attempts are being made to develop models suitable for studies of immunodepression induced by drug treatment and by tumor-associated factors in tumor-bearing hosts. Particularly a novel approach is being employed in an attempt to obtain new antigenic targets at the level of tumor cells through antigenic changes induced by drug treatment in animals transplanted with neoplastic cells.

Proposed Course of Project:

This section will continue to be involved in developing animal models for immunochemotherapy studies. These models will include studies with specific immunotherapy involving drug-induced antigenic changes as well as nonspecific immunotherapy with various immune stimulators.

The athymic (nude) mouse program will be further developed to grow and treat several human tumors of solid or lymphoid origin.

Further models will be developed for measuring immunosuppressant activity of antitumor agents and their relationship to chemotherapeutic response.

Keyword Descriptors:

Immunochemotherapy, immunosuppression, nude mice, tumor antigenicity

Publications:

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3. Campanile, F., Houchens, D., Gaston, M., Goldin, A., and Bonmassar, E.: Increased immunogenicity of two lymphoma lines following drug treatment in athymic (nude) mice. J. Nat. Cancer Inst. In press.

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6. Johnson, R., Houchens, D., Gaston, M., and Goldin, A.: Effects of levamisole (NSC 177023) and tetramisole (NSC 102063) in experimental tumor systems. Cancer Chemother. Rep. In press.

7. Kende, M., Keys, L., Gaston, M., and Goldin, A.: Immunochemotherapy of transplantable Moloney leukemia with cyclophosphamide and allogeneic spleen lymphocytes and reversal of graft-versus-host disease with alloantiserum. Cancer Res. 35: 346-351, 1975.

Project No. Z01 CM 07100-02
1. Laboratory of Experimental Chemotherapy
2. Tumor Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1974 through June 30, 1975

Project Title: Tumor Biology and Chemotherapy

Previous Serial Number: NCI 7100, Part of NCI 3575

Principal Investigator: Randall K. Johnson

Co-Principal Investigator: Abraham Goldin

Other Investigators: J. David Bessman, Artemio A. Ovejera, Toshio Inouye, Iwao Umezawa, Manik Chitnis, David P. Houchens

Cooperating Units: S. C. Cohen, Walter Reed Army Medical Center; H. L. Gabelnick, Division of Research Services, NIH; D. M. Levy, H. Metz, Biomedical Engineering and Instrumentation Branch, DRS, NIH; I. Kline and S. Vadlamudi, Microbiological Associates, Inc.; J. Meienhofer, Hoffmann-LaRoche, Inc.; E. Modest, T. S. A. Samy, Sidney Farber Cancer Center; M. A. Apple, University of California; G. Starke, Stanford University; E. Katz, Georgetown University; K. Gerzon, Eli Lilly Company; J. Levy, University of North Carolina

Man Years

Total: 8.5
Professional: 5.5
Others: 3
Plus animal care support from Mammalian Genetics and Animal Production Section, DR&D, DCT, NCI

Objectives:

To undertake fundamental studies in therapy of experimental tumors; to investigate potential and established clinical cancer chemotherapeutic agents with respect to their effect on malignant growth and selectivity; to determine the relationship between various biological properties of tumors and response to chemotherapy; to study structure-activity relationships among major classes of antineoplastic agents; to develop underlying principles of successful cancer therapy. Specific objectives include: study of drug sensitivity differences and control mechanisms in the cell cycle; study of the origin and basis of invasiveness, metastasis and tumor angiogenesis and the effects of drugs on these phenomena; investigation of means for effective therapy and prevention of emergence of resistant cell populations; study of the mechanism of drug action and mechanism of drug-induced cell death; study of the influence of drugs on hematopoietic and other sensitive host tissues in relation to antitumor effect; development of new and improved biological systems for screening and detailed evaluation of potential antineoplastic agents; determination of underlying principles of effective combination chemotherapy and combined modality treatment.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Structure-Activity Studied and Analog Evaluation

A. Actinomycins. (R. K. Johnson, J. Meienhofer, M. A. Apple, E. Modest, E. Katz and A. Goldin)

A major effort was initiated to find an actinomycin analogue with greater selectivity or a broader spectrum of activity than actinomycin D. Rigorous comparative evaluation of over twenty analogues is being undertaken in L1210, B16, and P388 which are minimally, moderately and highly sensitive, respectively, to the parent. It was found that in vivo activity does not correlate well with in vitro and antibacterial assays for activity. Analogues with amino acid substitutions in the 3'-position of the cyclic pentapeptide chains appear to offer the high potential for yielding compounds with improved therapeutic activity.

B. Anthracyclines. (R. K. Johnson and A. Goldin)

The cinerubins, anthracyclines with distinct differences in aglycone and sugar moieties compared to adriamycin, were studied in detail. Cinerubins A and B are not completely cross-resistant in P388 sublines resistant to adriamycin or daunomycin. Cinerubin A is active against L1210, P388 and B16 and Lewis lung carcinoma. Evaluation of cinerubin A for cardiotoxicity is underway.

C. Vinca Alkaloids. (R. K. Johnson, M. Chitnis, K. Gerzon and A. Goldin)

Two vinblastine amide derivatives, one of which is in clinical trial under a Lilly IND, are being compared to vincristine and vinblastine in a number of tumor systems. The drug in clinical trial was superior to the established agents against early and advanced B16 melanoma.

D. Cyclophosphamide Analogues. (I. Umezawa, R. K. Johnson and A. Goldin)

Two alkylsulfonate analogues of ifosfamide were tested in L1210 and Lewis lung and found to be clearly inferior to cyclophosphamide and ifosfamide.

II. Detailed Evaluation of New Potential Antitumor Agents

A. Phosphonacetyl-L-aspartic acid [PALA]. (T. Inouye, R. K. Johnson and G. Starke)

This rationally-synthesized antimetabolite, a transition-state inhibitor of aspartyl transcarbamylase, the initial and rate-limiting enzyme of de novo pyrimidine biosynthesis, was shown to be active against P388, B16 and Lewis lung, but not L1210.

B. Kidamycins. (I. Umezawa, R. K. Johnson and A. Goldin)

Kidamycin and acetylkidamycin represent a new class of antibiotics which interact with DNA. They were shown to have activity against P388 but not L1210, B16 or Lewis lung.

C. 2-Chloro-5-methylphenoxaphosphinate [CMPP]. (M. Chitnis, R. K. Johnson and J. Levy)

CMPP is a potential antitumor agent which may specifically interfere with metastasis. In the Lewis lung carcinoma CMPP reduced the number of lung metastases without affecting primary tumor growth or survival.

D. Neocarzinostatin [NCZ]. (R. K. Johnson, T. S. A. Samy and E. Modest)

Comparative chemical and biologic analysis of clinically formulated NCZ and a more extensively purified NCZ prepared at Sidney Farber Cancer Center revealed that the clinical material is about 10-fold more toxic to mice than the purified protein. The purified NCZ was also clearly superior in antitumor activity. Purification of the clinical material yielded three fractions which are undergoing concurrent chemical and biologic evaluation.

E. Non-specific Immune Stimulating Agents. (D. P. Houchens, R. K. Johnson and A. Goldin)

Levamisole and tetramisole were shown to be devoid of antitumor activity in syngeneic tumor systems but were shown to have a variable effect on primary viral-induced rhabdomyosarcoma, a system in which the immune response plays an important role. These agents did not reverse drug-induced immunosuppression in this viral-induced tumor system and are thus different in action from BCG. The clinical formulation of Corynebacterium parvum was shown to have definite activity against P388 leukemia both when used alone and in combination with adriamycin.

III. Resistance and Cross-Resistance to Antitumor Agents

A. Development of Sublines of P388 Leukemia Resistant to Adriamycin and Daunomycin. (R. K. Johnson and A. Goldin)

Two resistant sublines of P388 were developed by daily exposure to either adriamycin or daunomycin for several transplant generations. The cross-resistance patterns of P388/ADR was investigated. Cross-resistance was demonstrated to daunomycin and other related anthracyclines including rubidazone and carminomycin. Collateral sensitivity to a variety of alkylating agents (cyclophosphamide, melphalan, mitomycin C and cis Pt [II]) was found. Cross resistance was observed for a number of agents which interact with DNA (ellipticine, ICRF 159, actinomycin D, and mithramycin) as well as to several antineoplastic alkaloids (vincristine, vinblastine, VM 26, and emetine). Sensitivity to antimetabolites (5-FU, methotrexate and ara C) was retained.

B. Influence of Combination Chemotherapy on the Emergence of Resistance. (T. Inouye, R. K. Johnson and A. Goldin)

Resistance to ara C emerges rapidly in L1210. By adding an ineffective dose of cyclophosphamide to a daily treatment regimen of ara C we were able to delay the emergence of ara C resistance by four transplant generations. The relationship of this phenomenon to dose level is currently under investigation.

IV. Effects of Antitumor Agents on Wound Healing. (S. C. Cohen, H. L. Gabelnick, R. K. Johnson and A. Goldin)

As an extension of our studies with adriamycin and cyclophosphamide it was shown that therapeutic doses of diverse antitumor agents affected the strength of skin wounds in mice at different times of the wound healing process. Vincristine, methotrexate, and actinomycin D interfered with early phases of wound healing. BCNU inhibited all phases. Adriamycin and 5-FU had no affect. Bleomycin had a transient effect on an intermediate phase and cyclophosphamide had a specific effect on late phases of wound healing.

V. Growth Kinetics and Interactions of Primary and Metastatic Lewis Lung Carcinoma. (A. A. Ovejera, J. D. Bessman, R. K. Johnson and A. Goldin)

Excision of a primary intramuscular Lewis lung carcinoma results in more rapid growth of established lung metastases. Preliminary results indicate that daily injection of serum from primary tumor-bearing mice into animals with surgically excised tumors slows the growth of the lung metastases. When two primary tumors were implanted there was no influence of one tumor on the growth of the other. This is contrary to published reports with this tumor system.

VI. Use of the Coulter Channelyzer for Monitoring Erythrocyte Dynamics in Patients. (J. D. Bessman and R. K. Johnson)

Using this new cell sizing instrument we were able to demonstrate that anisocytosis was a common occurrence in hospitalized patients. An accurate identification was made of the mean corpuscular volume of the predominant erythrocyte population and changes in this parameter with therapy or disease progression were evaluated.

VII. Design of a Constant-Density Cell Culture Apparatus for Automated Biochemical and Cytokinetics Analysis. (R. K. Johnson and D. M. Levy and H. Metz)

A constant-density spinner culture apparatus was designed and is under construction. The instrument allows for long-term sampling and incubation of constant-density cell cultures with drugs or labelled precursors. The instrument is near completion and will be used to assess the effects of drugs in relation to the cell cycle and to relate cytotoxicity to biochemical events and to the primary pharmacologic effects of drugs on tumor cells.

Significance to Biomedical Research and the Program of the Institute:

As outlined in the Objectives, this project is designed to develop fundamental information on the principles of effective chemotherapy. Studies are initiated in response to current clinical needs. In addition, fundamental studies on animal tumor models are directly geared to the goals of the Drug Research and Development program of the DCT for improved screening and detailed evaluation of potential antineoplastic agents and combination regimens.

Proposed Course of Project:

As laboratory renovations are completed more emphasis will be placed on *in vitro* studies in tissue culture to evaluate drug effects and tumor biology. Emphasis will continue to be placed on structure-activity relationships among analogues of the most important clinical antitumor agents, resistance and cross-resistance to antitumor agents, and detailed evaluation of new agents with unique mechanisms of action. An extensive program of chemotherapy of human tumors in athymic mice will be initiated. During the coming year two new contracts will be initiated; the first will deal with development of experimental solid tumor systems for screening and drug evaluation, the second will be concerned with development and investigation of resistant tumor sublines *in vivo* and *in vitro*.

Keyword Descriptors:

Cancer chemotherapy, resistance, cross-resistance, structure-activity relationships, tumor growth kinetics.

Publications:

1. Bessman, J. D., and Johnson, R. K.: Erythrocyte volume distribution in normal and abnormal subjects. *Blood*. In press.

2. Cohen, S. C., Gabelnick, H. L., Johnson, R. K., and Goldin, A.: Effects of cyclophosphamide and adriamycin on the healing of surgical wounds in mice. *Cancer*. In press.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1974 - June 30, 1975

As one of the five major subdivisions of the Division of Cancer Treatment (DCT), Experimental Therapeutics (ET) has primary responsibility for an intramural research program and an extramural contract program concerned with the pharmacology, toxicology, and mode of action of cancer chemotherapeutic agents and other drugs used as single agents or in combined modalities. The program also conducts a major effort in molecular biology and biologic markers. Thus, studies within this program cover a broad range of drug-host phenomena at the whole body, cellular and molecular levels.

Organizationally, the intramural and contract research programs are shared among the Office of the Associate Director and four discrete but closely related laboratories: (1) Laboratory of Toxicology; (2) Laboratory of Chemical Pharmacology; (3) Laboratory of Molecular Pharmacology; and (4) Laboratory of Tumor Cell Biology.

Toxicological studies

The mission of the Laboratory of Toxicology (LT) is to initiate and maintain responsibility for a research program to assist the clinician in making an intelligent prediction as to the adverse reactions which may occur in patients receiving various antitumor agents during early clinical trials and to advise him on the steps which should be taken for their reversal. This is accomplished through large animal protocol toxicologic studies and special toxicity studies carried out by the contract mechanism and through an intramural laboratory research program employing various animal model systems for biochemical, metabolic, and histopathologic toxicological studies.

During the past year, a share in the responsibility for the supervision of large animal toxicologic studies was assumed by Battelle Memorial Institute which was awarded a Prime contract after extensive open competition. The LT will now receive much needed assistance in the summary and submission of toxicologic data for an Investigational New Drug Application (INDA) to the Food and Drug Administration before a Phase I Clinical Trial is initiated.

The Prime contract has been directed to carry out its mission under four tasks. Task I involves large animal (dog and monkey) protocol toxicity studies which are initiated through a limited number of subcontracts to enture timely performance of the studies and delivery of the protocol report for submission to the FDA as part of an INDA. Task I studies are currently underway or have been completed on 6 agents in the initial year of the Prime as shown in Table 1. Our estimate is that the capacity of the Prime this coming year will be 10 agents with a potential increase of up to 15 agents at the end of the year. The completion of a protocol study requires 8 months.

Task II also involves large animals but in abbreviated toxicity studies on agents for which data generated from foreign countries or other sources may be too incomplete to satisfy the FDA for filing an INDA. Both alkylating agents shown under Task II in Table 1, were submitted to the DCT Program as part of the U.S.-Soviet Union agreement to exchange drugs of interest to clinical cancer chemotherapy.

Task III is concerned with organ-specific toxicity studies, for example, the use of animal models for predicting the cardiac, renal, or pulmonary toxic potential of antitumor agents. Task IV involves the computerization of protocol toxicity data which will enable the Division to improve preclinical toxicity research, shorten lead times for protocol starts and improve responsiveness to the FDA, and inquiries from other biomedical sources.

The remaining antitumor agents shown in Table 1 are in toxicologic studies being conducted at contract sites other than that of the Prime contractor.

The laboratory research program intramurally continues to emphasize studies of the biochemical toxicology of oncolytic enzymes such as L-asparaginase and L-asparagine synthetase in cancer therapy. In this regard, L-asparagine synthetase may be of potential usefulness as a marker for tumor cell burden. In other studies the rabbit model has been refined to permit the systematic study of the cardiomyopathic potential of analogs of adriamycin and daunorubicin. The finding of calcium accumulation in the myocardia of adriamycin-treated rabbits, thus potentially altering myocardial membrane permeability, offers another clue as to the mechanism by which adriamycin is cardiotoxic. Initial studies on the pathologic effects of platinum analogs indicate that the rat may be a potentially useful model for assessing the renal toxicity of this unusual class of antitumor agents. The role of microsomal enzymes in the biotransformation of antitumor agents constitutes another major research area of the laboratory in its effort to study other pharmacological parameters which will enhance or decrease toxic reactions. These studies are being carried out _in vivo_ and _in vitro_ to systematically determine the drug metabolizing capability of pulmonary, renal, and hepatic systems. Various animal species are being studied to determine which species most closely resembles man in their ability to metabolize drugs.

Pharmacological studies

The Laboratory of Chemical Pharmacology (LCP) maintains primary responsibility through an intramural and contract research program for the development and use of analytical-biochemical methodology for studies of the pharmacological disposition, metabolism, and mechanism of action of antitumor agents in man, laboratory animals, and _in vitro_ predictive systems. The long-term objective is to permit the design of more effective therapeutic dose schedules, and drug combinations and other treatment modalities for the clinic.

Table 2 lists 25-30 agents which are currently under study at various pre-clinical and clinical stages of pharmacological evaluation. This is exclusive of the intramural research effort in which 5-10 agents are under study at any given time. Not included in Table 2 are pharmacological studies being considered during Phase I Clinical Trials which are sponsored through the contract mechanism by the Cancer Therapy Evaluation Program of the DCT.

The pharmacokinetics and biochemical pharmacology of the clinically active folic acid antagonists, methotrexate (MTX), continue to receive major attention in the laboratory by intramural investigators. To highlight some results of these studies: 1) Constant infusions of MTX in mice to determine the sensitivity of tumor and normal host target tissues, e.g., gastrointestinal epithelium and bone marrow, demonstrated that the rates of recovery of select biochemical pathways in these tissues may account for their differential sensitivities to the drug; 2) Intrathecally administered MTX in patients resulted in pharmacologically significant plasma levels ($>10^{-8}$M) than were observed in patients who had received the drug intravenously or by the oral route. These observations may possibly provide and rationale for enhanced bone marrow and gastrointestinal toxicity of MTX when the drug is given by the intrathecal route as compared to the oral and intravenous routes; 3) Pharmacokinetic studies with MTX were also performed in patients with ovarian cancer and in patients with osteogenic sarcoma and other malignancies followed by citrovorum rescue receiving 50 mg/kg or greater of the drug. The latter studies indicated that renal toxicity which is a significant risk following high dose levels of MTX, may possibly be averted through hydration and alkalinization procedures to prevent precipitation of the drug in the kidney tubules.

Studies of folate derivatives which achieve significant antitumor effects without inhibition of dihydrofolate reductase appear to be leading to a direct interface with the mechanisms by which citrovorum factor acts as an antidote for high-dose methotrexate.

New agents included in pharmacological disposition studies in pre-clinical animal systems and in man were, methanesulfon-m-anisidine, 4'-(9-acridinyl-9-amino)monohydrochloride, coralyne sulfoacetate, inosine dialdehyde and gallium. Maytansine, a new ansa macrolide plant alkaloid, tilorone hydrochloride and mouse interferon were examined for antitumor activity in selected transplantable sensitive and resistant rodent tumor systems as an adjunct to mechanism of action studies. In studies on macromolecular synthesis, maytansine appeared to have a greater inhibitory effect on DNA synthesis as compared to RNA and protein synthesis.

Depot forms of drugs such as cyclocytidine and ftorafur have focused attention on a very important aspect of drug delivery, namely the maintenance of prolonged blood levels after a single injection and in addition have revealed important pharmacodynamic effects unrelated to antitumor response.

Interaction of drugs in a cellular pharmacologic milieu have shown significant effects which may well influence their activity *in vivo* when administered in combination.

The Membrane Transport Section is actively pursuing studies to determine factors which affect the exchange of drugs and other molecules between blood, brain, and CSF and to establish guidelines which should be used in planning optimal routes and dosage schedules for the uptake and distribution of drugs to normal and neoplastic tissue of the CNS. During the past year, it was demonstrated among several findings that carotid (arterial) infusions of drugs are superior to intravascular infusions but only for drugs which are either rapidly and tightly bound to active sites within the CNS parenchyma or rapidly metabolized to active polar molecules retained by the tissue. Computer modelling has been applied extensively to the mathematical analysis of these phenomena and other vascular-CSF perfusion studies to better define the underlying pharmacological principles.

Molecular biology and pharmacology studies

An important scientific milestone in biomedical research was reached by the Laboratory of Tumor Cell Biology (LTCB) during the past year in its continuing efforts to gain a better understanding of the processes involved in the molecular control of cellular growth and differentiation, neoplastic transformation, and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for the control and eradication of human malignancies. This was a report of the culmination of carefully planned laboratory experiments in cell biology and virology by a group of NCI scientists led by Dr. Robert C. Gallo (Chief, LTCB), resulting in the isolation of a complete virus from the laboratory grown cells of a patient with acute myelogenous leukemia. In these investigations, Dr. Gallo and his colleagues have used biochemical and immunological techniques developed in their laboratory over the past five years and in laboratories of other scientists in the NCI and elsewhere. They have compared the RNA, reverse transcriptase (first shown by Gallo to be present in human leukemic cells but not in normal white cells) and certain antigens of this virus to components of known non-human primate cancer viruses and to viral components of the patient's fresh leukemic cells. A strikingly high degree of antigenic relatedness was demonstrated of the human virus to the gibbon ape and woolly monkey oncogenic type-C RNA viruses. While the human virus has not been proven to be a causative agent, the findings by Gallo and his associates argue for the possibility that the non-human primate viruses may be implicated in the leukemogenic process in man as a result of a very complex genetic and environmental setting.

Additional resources will be provided where necessary by the DCT as well as the Institute's viral oncology program (Division of Cancer Cause and Prevention) to quickly follow-up this important discovery to allow its confirmation by other laboratories within the NCI and elsewhere, to extend the work to the examination of other human leukemias for similar viral isolates and to provide biohazard containment facilities for production of the AML human virus. The latter will be accomplished primarily through the contract mechanism at the Frederick Cancer Research Center.

In pursuing its efforts to better understand the reactions of cancer chemotherapeutic agents and other model drugs with macromolecular targets within cells, the Laboratory of Molecular Pharmacology is using several well defined approaches. The first approach involves studies on the damage and excision repair of DNA using mouse L1210 cells and human fibroblasts grown in culture. In these studies a new technique has been developed which measures DNA single-strand breaks after exposure to pharmacologically active levels of drug and ultraviolet radiation in mammalian cells. Bleomycin, among a number of chemotherapeutic agents examined, was the most potent drug in its ability to product single-strand DNA breaks. Fibroblasts from patients with xeroderma pigmentosum, which lacks the excision repair mechanism, failed to exhibit DNA breaks after exposure to UV radiation and, to a smaller extent, after exposure to anthramycin. The results indicate that the excision repair system plays a role in the production of DNA breaks. The second approach involves studies of drug effects on replication of DNA, and finally, studies of potential cell culture models for chemotherapy are being pursued.

Biologic markers program

An intramural and contract research program continues to be developed in ET for the evaluation of test materials as potential biological markers to assess the tumor status, regression or progression for a patient with cancer. Frequently, the current clinical procedures for determining response or lack of response of a malignancy to therapeutic efforts are completely inadequate to appraise even major changes as they occur. Biochemical or biological materials, products of tumor cells, and present in body fluids of patients with cancer measured by appropriate analytical techniques could provide a sensitive means for evaluating antitumor response.

Specific analytical techniques, some newly developed, are being utilized by contractor investigators and intramural scientists for assessing such potential markers as t-RNA nucleosides, polyamines, β-amino isobutyric acid, and serum fucose/protein ratios in serums and urines of cancer patients' samples obtained from NCI's clinical services and several hospital centers throughout the United States. Immunological markers such as CEA (carcinoembryonic antigen), hCG (human chorionic gonadotropin) myeloma protein, α-fetoprotein, sialic acid (glycoproteins) and Regan's isoenzyme are also being studied as potential markers. Results of studies during the past year indicate that the levels of t-RNA nucleosides and polyamines are elevated in the urines of more than 50% of patients with several different types of solid tumors. CEA, hCG, and N^2,N^2-dimethylguanosine were elevated in 97% of advanced breast cancer patients.

The plan for the coming year is to expand this program considerably and negotiations are on-going at the Frederick Cancer Research Center to establish a Biological Marker Analytical and Research Facility. This facility would provide analyses of patient samples from selected clinical trials for potential biologic markers currently under study and include in-depth research resource in an effort to develop new, better, and more specific markers. Studies will be carried out to compare marker levels in body fluids to the current methods for assessing tumor status. Initially, the plan is to coordinate the acquisition of patients' samples between the NCI clinical services, the on-going Phase II study contracts in the Cancer Therapy Evaluation Program of the DCT, and with several major cancer centers in the United States. Research will include studies of all types of materials present in body fluids. A body fluid sample and data computer bank, to include patient and analytical results will also be established in order to determine, rapidly, the value of a new marker and to compare it with previously studied markers.

TABLE 1

ANTITUMOR AGENTS UNDER TOXICOLOGICAL EVALUATION: SUPPORTED BY CONTRACT

PROTOCOL TOXICOLOGY STUDIES - TASK I

ADRIAMYCIN (NSC-123127)
CHLOROZOTOCIN (NSC-178248)
CORALYNE SULFOACETATE (NSC-154890)
3-DEAZAURIDINE (NSC-126849)
INDICINE-N-OXIDE (NSC-132319)
MAYTANSINE (NSC-153858)

ABBREVIATED PROTOCOL TOXICOLOGY STUDIES - TASK II

ASALEY (NSC-167780)
FLUORODOPAN (NSC-73754)

SPECIAL TOXICOLOGICAL STUDIES

EFFECTS ON REPRODUCTION

ADRIAMYCIN (NSC-123127)
BCNU (NSC-409962)
CCNU (NSC-79037)
MeCCNU (NSC-95441)
DTIC (NSC-45388)
STREPTOZOTOCIN (NSC-85998)

CARDIOTOXIC EFFECTS

ADRIAMYCIN (NSC-123127)
DAUNORUBICIN (NSC-82151)
ADRIAMYCIN-DNA COMPLEX (NSC-169534)
DAUNORUBICIN-DNA COMPLEX (NSC-169533)
CARMINOMYCIN (NSC-180024)
RUBIDAZONE (NSC-164011)

TOXIC EFFECTS OF DRUG COMBINATIONS

CYTOXAN (NSC-26271) PLUS ADRIAMYCIN (NSC-123127)
5-FLUOROURACIL (NSC-19893) PLUS MELPHALAN (NSC-8806)

TABLE 2

ANTITUMOR AGENTS UNDER PHARMACOLOGICAL EVALUATION: SUPPORTED BY CONTRACT

PRECLINICAL PHARMACOLOGICAL STUDIES

ALKYLATING AGENTS

- BCNU (NSC-409962)
- CCNU (NSC-79037)
- MeCCNU (NSC-95441)
- CHLOROAMBUCIL (NSC-3088)
- MELPHALAN (NSC-8806)
- IMIDAZOLE CARBOXAMIDE, DI-METHYL TRIAZENO: DTIC (NSC-45388)
- PROPANOL, IMINODI-DIMETHANE SULFONATE (ESTER) HYDROCHLORIDE: YOSHI (NSC-102627)

ANTIMETABOLITES

- 5-AZACYTIDINE (NSC-102816)
- METHOTREXATE ESTERS
- TRIAZINATE: BAKER'S ANTIFOLATE (NSC-139105)

NATURAL PRODUCTS

- ADRIAMYCIN (NSC-123127)
- DAUNORUBICIN (NSC-82151)
- ELLIPTICINE (NSC-71795)
- INDICINE-N-OXIDE (NSC-132319)
- STREPTOZOTOCIN (NSC-85998)

MISCELLANEOUS

- CYTEMBENA (NSC-104801)
- DICHLOROALLYL LAWSONE (NSC-126771)
- GALLIUM NITRATE (NSC-15200)
- 5-HYDROXYPICOLINALDEHYDE THIOSEMICARBIZONE (NSC-107392)
- ICRF-159 (NSC-129943)
- LAPACHOL (NSC-11905)

CLINICAL PHARMACOLOGY STUDIES

ALKYLATING AGENTS

- ASALEY (NSC-167780)
- MELPHALAN (NSC-8806)
- YOSHI (NSC-102627)

ANTIMETABOLITES

- 5-AZACYTIDINE (NSC-102816)
- CYCLOCYTIDINE (NSC-145668)
- 5-FLUOROURACIL (NSC-19893)
- FTORAFUR (NSC-148958)
- METHOTREXATE (NSC-740)

NATURAL PRODUCTS

- STREPTOZOTOCIN (NSC-85998)

MISCELLANEOUS

- CYTEMBENA (NSC-104801)
- DICHLOROALLYL LAWSONE (NSC-126771)

ANNUAL REPORT

OFFICE OF THE ASSOCIATE DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

NATIONAL CANCER INSTITUTE

July 1, 1974 -- June 30, 1975

Studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in several species of animals have been undertaken. Intravascular infusions, ventriculo-cisternal perfusions, lumbo-cisternal perfusions, ventricular injections, theoretical and mathematical analysis, plus computer modelling have been the methods generally employed.

From our experimental theoretical, and computer modelling work, we are formulating the principles which are involved in the exchange of materials between blood, brain, and CSF and the guidelines which should be used in planning optimal route and dosage schedules for the uptake and distribution of drugs to normal and neoplastic neural tissue. To obtain the most uniform and wide-spread distribution of drugs to neural tissue, CSF, and meninges, the compound should be introduced into the blood stream; yet for this route of delivery to provide adequate tissue uptake, the drug must be either lipid soluble enough to passively diffuse across the blood-brain barrier or structurally resemble one of the types of molecules which are moved across the blood-brain barrier by special transport (carrier) systems. On the other hand, compounds which do not readily cross the blood-brain barrier may be delivered to the meninges and parts of the brain and spinal cord by way of the CSF. There are significant distribution problems, such as adequate mixing throughout the CSF compartment and depth of tissue penetration, however, when this route of administration is used to deliver drug to CNS tissue. Before infusing the drug into the CSF, consideration must be given to the site and manner of administration, the location of the tissue target site, and the transport properties of the drug. Our studies of arterial (carotid) infusions as a means of increasing the uptake of drugs by the central nervous system (CNS) and CSF indicate that this method of drug administration will only be effective in very specific circumstances. We have concluded from this work that intracarotid injections or infusions are superior to intravascular infusions only for a drug which is quickly and tightly bound to active sites within the CNS parenchyma, or is rapidly metabolized within the tissue to a polar, active form which is retained by the tissue, or is exceedingly rapid in its action against the neoplastic cells.

Using a double-indicator uptake technique, regional differences in blood flow and capillary permeability have been found within the brain and spinal cord of the cat. This work shows that significantly higher (3-5 times) rates of blood flow occur in areas such as the cerebral and cerebellar cortex than in other regions such as the pons and the spinal cord. For many compounds sizeable regional differences in the kinetics of molecular exchange exist within the CNS because of the differences in the rates of blood flow. From these results and our ventriculocisternal perfusion work, we are also finding some differences in capillary permeability (the blood-brain barrier) between various areas of the CNS.

Our ventriculocisternal studies in dogs and monkeys have enabled us to develop a fairly complete model which describes the movements of materials between blood, brain extracellular fluid, brain cells, and CSF. Using this model and reasonable values for the various transport steps, we determined that the CSF can only serve as an effective buffer or sink for brain fluids, if the brain capillaries are virtually impermeable to the substance of interest. Thus, if effective regulation of brain fluid composition is to occur, the brain capillaries must be functionally impermeable either by passive (tight membranes and junctions) or active (pumps and carrier systems) means. This model also predicts that studies of brain metabolism which use CSF as an indicator of CNS production rates may be greatly in error if the metabolite exchanges across brain capillaries at even a modest rate.

An examination of the lumbo-cisternal exchange of material between CSF and spinal cord was begun using a perfusion system in monkeys. It was determined from these studies that the permeability of the pia-glial membrane of the spinal cord was similar to that of the ependyma and that moderately sized (300-400 molecular weight) water soluble compounds exchange readily across the membrane. Estimates of the size of the spinal cord's extracellular space yielded values of 13-18%. The rate of tracer compound equilibration within the spinal cord extracellular fluid compartment was consistent with the diffusional exchanges rates previously reported from this laboratory for the caudate nucleus and cerebral cortex. Our measurements of cerebrospinal fluid production within the spinal cord subarachnoid space indicated that little CSF is formed here. Analysis of brain cellular and capillary permeability, as in ventriculocisternal perfusion studies, can also be made with this technique. Such measurements are currently being made with various tracer solutes.

From our comparative studies, a wide range of observations have been made. The rate of CSF production, the flow patterns, the CSF absorption capacity, and the sites of CSF absorption were investigated and described for the dogfish shark. Using the in vitro choroid plexus preparation of Rall and Sheldon, the kinetics of glucose transport of this tissue were studied. Evidence of transport saturation, definite sterospecificity, and transport competition were found. The movements of a series of polar compounds from blood to CSF and several regions of the brain and spinal cord were examined in the dogfish shark. From this work measurements of CNS and choroid plexus blood flow were made and an unusual transport system, which mediates the exchange of urea between blood and CSF, was found. Ventricular perfusion studies indicated the similarity of the blood-brain barrier in this species to a tight epithelial or cellular membrane.

Project No. Z01 CM 03500-12 EXT
1. Office of the Associate Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

Previous Serial Number: NCI-3500

Principal Investigator: Joseph D. Fenstermacher

Other Investigators: Ronald Blasberg, Warren Lux, John Kessler, Clifford Patlak, Anthony Guarino, Leon Sokoloff, Arnost Kleinzeller (non-NIH), Andres Roomet (non-NIH), Walter Eckman (non-NIH), and Alan Cowles (non-NIH).

Cooperating Units: Biometrics Branch, NIMH; Laboratory of Cerebral Metabolism, NIMH; Laboratory of Toxicology, DCT/NCI; Department of Physiology, University of Pennsylvania; Department of Neurology, University of Vermont; Department of Neurosurgery, University of Western Ontario; Computer Center, University of Missouri.

Man Years:

Total:	6.5
Professional:	4.0
Others:	2.5

Project Description

Objectives:

The objectives of this project are to quantify the transport of various materials of physiological and pharmacological importance between blood, neural tissue, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to develop methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The method of ventriculocisternal perfusion of various radioactive materials and subsequent brain tissue sampling has been utilized in monkeys, dogs, and dogfish sharks for measurements of brain capillary and cellular permeability. Furthermore a spinal cord perfusion technique has been developed and employed for similar studies in the spinal cord. An experimental system employing simultaneous intracarotid and intravenous infusions has been devised and used for assessing the effectiveness of these routes of drug administration. Studies of brain and CSF uptake and distribution of intravenously administered, radioactively labeled physiological and pharmacological materials have been undertaken in dogs, monkeys, and elasmobranchs. Finally, a technique for studying the growth of regenerating optic nerves and brain (optic tectum) in goldfish is being developed.

Major Findings:

From our theoretical, experimental, and computer-modelling work, certain guidelines for effective delivery of drugs to the central nervous system (CNS), CSF, and meninges have emerged. To obtain the most uniform and widespread distribution of drug to the CNS as well as the CSF and meninges, the material should be introduced intravenously; yet for this route of delivery to provide adequate tissue uptake, the drug must be either lipid soluble enough to passively diffuse across the blood-brain barrier or structurally resemble one of the types of molecules which are transported across the blood-brain barrier by special (carrier) systems. On the other hand, compounds which do not readily cross the blood-brain barrier may be delivered to the meninges and surface portions of the brain and spinal cord by way of the CSF. There are, however, significant problems in drug delivery such as adequate mixing throughout the CSF compartment and depth of tissue penetration when this route of drug administration is used. Before infusing the drug into the CSF, consideration must be given to the site and manner of administration, the location of the tissue target site, and the transport properties of the compound. Antineoplastic agents should be infused by the arterial route, usually the carotid artery, when it is important to achieve a high initial or peak concentration in the brain, when the drug has a high affinity for the target organ or for all tissues, or when the drug is rapidly metabolized. From this work we have shown that the considerable advantage of increased drug delivery to the CNS obtained during the initial or first passage of arterially injected drug is rapidly dissipated if the material continues to recirculate in significant amounts, even for relatively short periods of time.

Using a double-indicator (tri-iodo-fluoro-methane and antipyrine) uptake technique, regional differences in blood flow and capillary permeability have been found within the CNS of the cat. This work shows that significantly higher (3-5 times) rates of blood flow occur in areas such as the cerebral and cerebellar cortex and caudate nucleus than in other regions such as the pons, periventricular white matter, and spinal cord. For highly permeable compounds sizeable regional differences in the kinetics of molecular exchange between blood and tissue exist because of these differences in the rates of blood flow. From these results plus some of our ventriculocisternal perfusion studies, we are also finding some differences in capillary permeability (the blood-brain barrier) between various areas of the CNS. Such differences would cause variations in the regional uptake of drugs and regional effectiveness of chemotherapy.

Our ventriculocisternal perfusion studies in dogs and monkeys have fairly clearly characterized the kinetics and nature of material flows between the CNS and CSF and their dependence on ependymal, brain cellular, and brain capillary permeability. On the basis of these studies and a theoretical model of the system, we have determined that the CSF can serve as an effective buffer to brain fluid changes in ionic and molecular composition only if the permeability of the brain capillaries to these materials is very low. If the capillary permeability is significant (half-time of exchange less than 3 hours), only those tissue areas immediately adjacent (0.1-0.2 cm) to the CSF will be affected or buffered by the CSF. An examination of a related problem, the clearance from the CNS by the CSF of metabolites which have been produced by neural tissue, shows that the removal of such materials via this route strongly depends on the permeability of the brain capillaries. Only a very small portion of the total production of a particular metabolite will reach the CSF if the CNS capillaries are moderately to highly permeable to the metabolite. This finding casts a good deal of suspicion on a number of studies in which the rate of CNS production of a particular material has been estimated on the basis of its appearance in the CSF. Increases or decreases in the CSF levels of various metabolic end-products can be the result of changes in either capillary permeability or cellular metabolism.

An examination of the exchange of material between the CSF and the brain and spinal cord of monkeys was made using our technique of cisterno-lumbar perfusion. From this work we determined that the permeability of the spinal cord's pia-glial covering to sucrose and EDTA-Ca was similar to that of the brain's ependymal and pia-glial membranes, and that these molecules exchange readily between CSF and spinal cord. Using the equilibrium sucrose and EDTA-Ca spinal cord to CSF ratios (they were virtually identical), extracellular spaces (ECS) of 13.5% and 18% for spinal white and gray matter, respectively, were measured. The rates of tracer compound equilibration within the spinal cord's ECS were comparable with the diffusional exchanges previously reported from this laboratory for the caudate nucleus and cerebral cortex. Using this same technique, CSF production by periventricular brain tissue and spinal cord were measured by blue dextran dilution. The results indicate that the rate of CSF formation in the monkey is about 30 μl/min and that little or no CSF is produced by the spinal cord. Capillary and cellular exchange rates of several amine metabolites have also been made. These are the first precise measurements of spinal cord transport parameters and rates which have been made to date.

From our comparative studies, a wide range of observations have been made. Ventricular fluid (CSF) production rates were measured by ventricular perfusion in the spiny dogfish and found to be about .002 ml/min. In this species the resistance to bulk absorption of CSF was determined to be similar to that in mammalian species such as the rabbit and monkey; the cranial nerves and spinal canal seem to be the major pathways of CSF absorption. Using the *in vitro* choroid plexus preparation of Rall and Sheldon, the kinetics of glucose transport of this tissue were studied. Evidence of transport saturation, stereospecificity, and competition were found. The movements of a series of polar compounds from blood to CSF and several general regions of the CNS were examined in the dogfish shark. From the water exchange data rates of blood flow were found ranging from 2.2 ml/min-gm tissue for the choroid plexus to 0.04 ml/min-gm tissue for the spinal cord. The movement of urea from blood to CSF was surprisingly rapid and suggested that the flow of this compound between these two fluids is mediated by a special transport mechanism located at the choroid plexuses. Ventricular perfusion studies indicated the "tight" cellular nature of the blood-brain barrier in these creatures. Those materials which cross cellular membranes readily, e.g. water and ethanol, do the same at the dogfish blood-brain barrier; a similar parallelism was found for moderately permeable materials, such as acetamide and thiourea, and relatively impermeable compounds, such as mannitol and sucrose.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the brain, spinal cord, and meninges. Anti-tumor agents can be delivered to these tissues by the vascular and/or the cerebrospinal fluid systems; however, each of these two systems appears to have certain limitations as a means of effective drug delivery. Basic information concerning the transport physiology and movement of material into normal and neoplastic CNS and meningeal tissue from the blood and from the CSF is needed for the design and development of effective brain tumor chemotherapy protocols. Such information about blood-brain-CSF transport coupled with a knowledge of the physiochemical and pharmacological properties of specific antitumor drugs will enable the chemotherapist to choose the best route, manner, and timing for effective drug delivery to CNS and meningeal lesions. Basic knowledge of the transport of ions, metabolites, and other materials between blood, brain and CSF is essential to the understanding of the activities and functions of the central nervous system. Since our work is fundamental in nature, it is relevant to work being carried out by many other groups of bio-medical researchers, and important for an understanding of both normal and pathological brain and spinal cord processes. The type of work performed in our laboratory on this subject is, thus, meaningful and significant to the neurologist, the neuroncologist, the neurosurgeon, and the neuropathologist as well as the neurophysiologist, neurochemist, and neuropharmacologist.

Proposed Course:

1. Continue our studies of the transport of various physiological and pharmacological materials between blood, brain, and CSF and more precisely quantitate the permeability of brain capillary and cellular membranes.

2. Investigate further the formation, flow, and absorption of cerebrospinal fluid by and through CNS tissue.

3. Examine the effects of high and low dietary levels of vitamin A on CSF production, absorption, and pressure and on brain cell and capillary permeability of the rhesus monkey.

4. Determine the blood-spinal cord-CSF exchange parameters of various physiological and pharmacological agents.

5. Study regeneration, control systems, and growth factor(s) in the goldfish central nervous system.

6. Investigate, when appropriate fresh tissue is made available from either human or laboratory animal sources, the transport characteristics of various types of CNS tumors.

7. Study fluid and electrolyte flows under the pathological conditions of cerebral edema and hydrocephalus.

8. Further examine the peculiar blood to CSF movement of urea and other amides in elasmobranchs.

9. Continue our study of the transport of organic compounds by the dogfish choroid plexus *in vitro*.

10. Investigate the effects of various transport inhibitors such as amiloride and phloretin on the blood-brain-CSF exchange of ions and amides in elasmobranchs.

Keyword Descriptors:

Drug distribution, material transport, blood-brain barrier, cerebrospinal fluid, brain tumor chemotherapy, arterial (carotid) infusions, intrathecal chemotherapy, cerebral blood flow, brain capillary permeability, spinal cord, central nervous system, brain extracellular space, spinal cord extracellular space, comparative physiology, elasmobranchs, choroid plexus, nerve regeneration, axonal transport, neurophysiology, neuropharmacology, neurochemistry.

Honors and Awards:

None

Publications:

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Fenstermacher, J.D.: Mechanisms of Ion Distribution Between Blood and Brain. In Brady, R. (Ed.): National Institute of Neurological Diseases and Stroke Twenty-fifth Anniversary Volume, Basic Neuroscience Section. New York, Plenum (in press).

Project No. Z01 CM 03516-10 EXT
1. Office of Associate Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: NCI-3516

Principal Investigator: J.A.R. Mead

Other Investigators: V. T. Oliverio, M. C. Castle and D. A. Margileth, NCI; A. E. Auletta, and L. C. Mishra, Microbiological Associates, Inc.; J. P. Kutney, University of British Columbia; R. H. Liss, Arthur D. Little, Inc.; L. B. Mellett and W. Galbraith, Southern Research Institute; M. Friedkin, University of California San Diego.

Cooperating Units: None

Man Years:

Total	6
Professional:	4
Other:	2

Project Description:

Objectives:

To investigate the biochemical pharmacology and pharmacodynamics of new or established antitumor agents to better define the important parameters related to their chemotherapeutic activity.

Methods Employed:

High pressure liquid chromatography and standard laboratory procedures were employed.

Major Findings:

The studies in this project can be categorized under the following headings:

A. Folate Derivatives
B. Quinaldine
C. Cyclocytidine
D. Vincristine

E. Fluorinated Pyrimidines
F. Phenylalanine Mustard

A. Folate Derivatives

The antifolate 5-methyl-dl-tetrahydrohomofolate has been further studied in an attempt to delineate the biochemical basis for its selective action against methotrexate-resistant leukemia. Its lack of substantial effect, after a single dose, on the incorporation of labeled precursors into the DNA of target tissues characteristically susceptible to the effects of antifolates such as methotrexate is not readily explained. Work from other laboratories suggests that an important determinant of biological activity may well be the presence of the enzyme methylcobalamin transferase in sensitive tissues. This enzyme would thus convert 5-methyltetrahydrohomofolate to tetrahydrohomofolate which would be the proximate therapeutic agent. The amount of tetrahydrohomofolate formed would presumably be related to the level of enzyme in the tissues and could be assessed by measuring the appearance of C^1 units in the tissues. Radioactive drug (5-methyl-C^{14}-tetrahydrohomofolate) will be studied *in vitro* and *in vivo* to determine the relative lability of the methyl group. If 5-methyl-tetrahydrohomofolate acts as a depot form of tetrahydrohomofolate, it will be yet another example of the bioactivation of an antitumor agent.

Another folate derivative, which is really not an analog, that we have studied is N_{10}-formylfolate. This compound which can be formed *in vivo* in rat liver after administration of folate is a potent inhibitor of dihydrofolate reductase *in vitro*. The question was raised as to whether this would inhibit the growth of leukemia L1210 *in vivo*. The only previous test was several years ago when it was administered to mice late in the course of the disease and was judged inactive. An evaluation of its antileukemic effect against early L1210 is presently underway and an assessment will be made of its ability to influence the incorporation of radioactive precursors into DNA and inhibit dihydrofolate reductase *in vivo*.

B. Quinaldine

This drug has been studied from the standpoint of its distribution *in vivo* both as a suspension and as a specially formulated acetate derivative. The major part of the drug injected i.p. remained in the peritoneal cavity for up to 90 days and while the acetate derivative was cleared within 14 days, there was no therapeutic advantage to this formulation. Since it now appears that this drug achieves its prolongation of lifespan in leukemic mice at the expense of considerable toxicity, more than 50% mice die from toxicity at the optimum dose, this drug is not now under active consideration as a candidate for further development.

C. Cyclocytidine

This interesting derivative of arabinosyl cytosine, continues to reveal some very interesting pharmacological properties. It had been noticed earlier that this drug was localized extensively in the parotid salivary gland. It was therefore not difficult to make the association with the clinical syndrome of parotid pain. The pharmacodynamic basis for this effect remained obscure until recently. Serial electron micrographs of the mouse parotid gland taken at intervals after the drug revealed progressive changes within the glandular structure. Starting after about two hours there was a steady reduction in the zymogen granule content to zero in about 4-6 hours. There was then an accumulation of mucus within the gland which persisted until about 8 hours at which time there gradually appeared a series of attenuated zymogen granules. If a parallel series of events occurs in man, the engorgement with mucus would certainly explain the parotid pain.

In a somewhat different approach the composition of the salivary outflow was analysed after administration of the drug and found to reflect the changes suggested in the electron micrographic studies. Perhaps the most important aspect of this study was the fact that it was possible to block the effect with propanolol and dibenzyline. Whether this observation would be useful for the control of clinical side-effects remains to be seen.

It was logical to extend these studies to a very closely related derivative, 5-fluorocyclocytidine. The results showed again what changes can ensue in the pharmacological effect of a drug by substituting a halogen atom. 5-fluorocyclocytidine was devoid of effect on the ultrastructure of the salivary gland or its outflow.

The basis for the pharmacologic effect of cyclocytidine will be investigated further in subsequent studies.

D. Vincristine

A satisfactory method for the separation of vincristine from its decomposition products and/or metabolites has been developed. It involves reverse phase high pressure liquid chromatography with gradient elution. This method has been successfully used to prepare ultrapure ^{3}H-vincristine which has in turn been used to study the pharmacologic distribution of the drug in rats and dogs. Special emphasis has been given to the excretion of drug-derived radioactivity in the bile of both species where there is now good evidence for metabolic products of the drug which are different in the two species. It is intended that sufficient of these materials be isolated to permit their characterization.

In other studies the uptake of vincristine by leukemia L1210 and P388 cells has been investigated. Particular emphasis has been placed on the effects of other agents on this process. It was found, for example, that maytansine at high concentrations was inhibitory to the membrane transport of vincristine.

E. Fluorinated Pyrimidines

The relationship of the Russian drug, Ftorafur, to 5-FU and its biochemically derived metabolites is the subject of some speculation since it appears that the former may be converted to the latter *in vivo*. A high pressure liquid chromatographic technique has been developed to separate these materials and 5-FUDR. In conjunction with labeled drug the extent of this postulated conversion is being investigated in normal and tumor bearing mice.

F. L-Phenylanine Mustard

Very little definitive information is available concerning the pharmacologic disposition of this commonly-used clinical antitumor agent. Radioactive drug which is now available will be used to conduct these studies. High pressure liquid chromatography has been shown to provide a good technique for separation of parent drug and the chemical products formed by its interaction with common laboratory solvents and will be used for the separation of any biotransformation products.

Proposed Course of Project

The project will continue to follow a course aimed towards the acquisition of relevant pharmacological, toxicological and biochemical data for input into the Linear Array for drug development. Concurrently, phenomena of a basic nature will be investigated inasmuch as they are related to the physiological effects, in animals, of experimental antitumor agents.

Keyword Descriptors:

Reverse-phase high pressure liquid chromatography, 5-methyltetrahydrohomofolate, 10-formyl folate, cytosine arabinoside, cyclocytidine, ftorafur, L-phenylalanine mustard, vincristine, maytansine, dihydrofolate reductase, propanolol, dibenzyline, quinaldine.

Honors and Awards:

United States Patent 3,856,959 awarded to J.A.R. Mead, December 24, 1974, entitled, "Inhibition of Leukemia Utilizing 5-methyltetrahydrohomofolate."

Publications:

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Mead, J.A.R.: Biochemical Pharmacology and Drug Design. In Proceedings of the 27th Annual Symposium on Fundamental Cancer Research, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute: Pharmacological Basis of Cancer Chemotherapy, Houston, Texas, 1975, pp. 197-213.

SUMMARY REPORT
LABORATORY OF TOXICOLOGY
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

July 1, 1974 to June 30, 1975

I. Introduction

The Laboratory of Toxicology continues in its responsibility for supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. This important role has actually been carried out in the past by a rather diffuse contract mechanism, with the Laboratory of Toxicology retaining direct responsibility to supervise these studies, summarize the information, and aid in the submission of an INDA to the Food and Drug Administration before a Phase I Clinical Trial is initiated. In February of 1974, the Division awarded a prime contract, wherein the responsibility for these studies is shared by the Prime Contractor (Battelle Memorial Institute) and this Laboratory. Undoubtedly this Prime Contract made available more time for the inhouse staff to more properly pursue some important aspects of research into toxicological mechanisms of action caused by antineoplastic agents. For the next fiscal year the Laboratory is committed to two major goals: 1) to continue the improvement of the inhouse staff efforts in the area of basic research; and 2) to cooperate with the Prime Contractor in maintaining the good technical momentum it started during its first contract year. It is felt that with the continuing limitation of staff, it would be unrealistic to project any additional goals. On the other hand, if staff conditions are improved it is the strong desire of the Laboratory to define and support research in the area of delineating mechanisms of toxicity of anticancer agents with the hope of proposing remedies for these toxic actions.

Attempts to increase the level of excellence of the senior staff have been helped, on the one hand, by the Prime Contract relieving them of the daily supervisory chores which drained on their time. On the other hand, the failure to replace the 20% loss of positions which has occurred over the past 2 to 3 years in this Laboratory, continues to be a major detriment to growth. It is our belief that without growth, there can only be stagnation. In a desperate effort to infuse some "new blood" into this potentially stagnant situation, we have recently entered into a new area for us, i.e. recruitment of foreign fellows via mechanisms which do not count against personnel ceilings. Of course, such individuals are not expected to help with the Program activities of the Laboratory, but rather it is hoped that their presence will help in the basic research areas and in improving the sagging morale of the senior staff.

For the second year in a row, some senior investigators have sustained superior research efforts, in the face of such personnel losses, only by heroic personal effort. It was predicted in last year's annual report, that it was "unfair and unhealthy to expect this to continue." In all honesty it must be admitted that there is evidence of attenuation of these heroic personal efforts.

For the first time in our recent history we have had to prematurely terminate some projects due to staffing limitations. The management cry to "establish new priorities" but "within present resources" carries with it grave risks.

The risk is that you may terminate a highly worthwhile project because the potential is not currently recognized. Setting priorities in biomedical science simply has not yet achieved the fine state of art as found in the engineering and technological areas. In contemporary biomedical science, setting priorities is guess work, and no matter how much information you put into a "guess", you are going to be wrong a high percentage of the time. We pray we have not terminated the wrong projects.

While we have emphasized the positive aspects of the Prime Contract, it must be recognized that some features of this effort have been unpredictable and represented a greater time burden for some individuals than had been predicted. For example, it must be pointed out that the Prime Contract takes a different kind of effort because of its "incentive" features. In other words, because the contractor is receiving a portion of his fee from this mechanism, extensive documentation is required to justify the award or denial of fee. It has been very difficult to structure the roles of the members of the Laboratory in this award-fee-situation. It has been recommended that there be no overlapping roles between the persons requesting the work and the individuals recommending the award of funds as judged by performance of this same work. Thus three categories of personnel are required for proper operation of the Prime Contract: 1) compound officers, 2) contract monitors and 3) evaluators. Hence, it became obvious this year, that with the Laboratory down to five to six senior staff slots, the requirements that the three roles not overlap, was impossible to meet. Therefore, we were obliged to functionally delete two contract monitors.

We continue to insist, as we have in the past annual reports, that the supervision of contractors be done by members of the scientific staff who have considerable first hand knowledge about the research problems involved with the use of our often uniquely toxic antineoplastic agents. If the internal staff can not maintain and grow in this kind of knowledge, then some important observations by contractors can not be properly assessed. For example, while some contract effort is being expended in defining the cardiac toxicity of Adriamycin, the possible involvement of other organs which may have primary or secondary etiologic contributions has been investigated. Thus alterations have been demonstrated by the inhouse staff implicating kidney, bone, intestine, and liver as possibly contributory to the cardiotoxicity of this agent. The degree of emphasis on basic research progress was a turn up a new road as far as the Laboratory is concerned and during this third year of such a policy it would seem that considering the personnel cuts, some progress continues. Evidence for this will be seen in the individual reports following, where it will be particularly noted that several projects have been brought to logical conclusions and submitted for publication.

II. Internal Research Programs

The independent projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, and when toxicity does occur, what steps may be taken to reverse it.

A. Biochemistry Unit

In the past year, the Biochemistry Unit has continued its involvement with several projects related to the biochemical, pharmacologic and toxicologic properties of selected oncolytic enzymes. This group also has been involved with studies of the biochemical toxicology of nitrosoureas. One project with L-asparaginase was prompted by the numerous recent studies showing that different methods for "packaging" a drug can lead to substantial differences in uptake, distribution and presumably toxicity. Two recent relevant examples of this nature have appeared: one of these entails the use of liposomes (Lancet, June 29, 1974, p. 1313); a second utilizes DNA complexes of drugs such as adriamycin (Nature New Biol. 239, 1972, p. 110). The rationale for the use of such carriers is that they might make the administered drug less toxic to normal tissue and more toxic to neoplastic tissues. Hence, it has been reported (Biochemical Journal, 140, 1974, p. 323) that many of the undesirable effects inherent in the direct administration of drugs or enzymes can be circumvented by trapping them in liposomes before use. For the present study, erythrocytes were employed as "packages" for L-asparaginase or crude L-asparagine synthetase. The enzymes were forced into the cytoplasm of mouse red blood cells whose pore size was transitorily enlarged by exposure to cold hypotonic solutions. In general, the half-life *in vivo* of erythrocytes treated in this way was shorter-than-normal. However, interiorized L-asparaginase did prove to be catalytically active and capable of eradicating L-asparagine from the plasma compartment. These preliminary studies will be the subject of more intensive effort in the next fiscal year. As in the past several years, studies of the biochemistry and inhibition of L-asparaginase and L-asparagine have continued. The major rationale for the heavy emphasis in this area has been that the resistance to L-asparaginase appears to correlate with the presence of elevated activity of L-asparagine synthetase in the tumor cell. Interruption of the synthesis of L-asparagine appears to be one method to render such cells sensitive once again to L-asparaginase therapy. In addition to the obvious therapeutic potential of such manipulations of enzymes, substrates, and inhibitors, these efforts are of profound biochemical significance. While the present therapeutic applicability of oncolytic enzymes is not widespread, these ongoing studies focus on the important basic concepts of the mechanisms of tumor-resistance, a phenomenon that has led to many drug failures in the clinic. In order to conduct an indepth biochemical and kinetic examination of these concepts, it is imperative that purified enzymes be available. Thus, in this past year, emphasis has been placed on purification of L-asparagine synthetase. Toward this end, a variety of classical and contemporary purification techniques have been employed. At the present stage, about a one thousand-fold purification of the crude homogenate has been achieved. In addition to the wide spectrum of inhibitors of L-asparagine synthetase which were discussed in last year's annual report, present investigations have centered around the use of inhibitors such as metallic ions; it was found that, in addition to copper and cobalt, some endogenous ions such as calcium and zinc significantly inhibited the synthesis of L-asparagine *in vitro*. In addition, the nature of the endogenous inhibitor(s) of L-asparagine synthetase has been elucidated by the use of specific counter-agents such as EDTA, protamine sulfate, ammonium sulfate, trypsin and dithiothreitol. In recent studies on L-asparagine synthetase, this enzyme was found to be of value as a biologic marker of neoplasia. Hence, this enzyme was found to appear in the serum seven days after implantation of Leukemia 5178Y/AR (L-asparaginase resistant). Furthermore, the levels of this enzyme in serum increased as the tumor grew and metastasized. Like many other tissue-specific enzymes (e.g. creatine phosphokinase (CPK) from cardiac and skeletal muscle), this enzyme was found to be rather rapidly cleared from the serum with a half-life of about one hour. Levels of L-asparagine synthetase were found to increase also in the liver, spleen, kidney and other tissues concomitant with metastasis in mice tumored with Leukemia 5178Y/AR. The obvious extension of these studies to clinical serum samples has been initiated with the cooperation of two clinical groups. A sensitive assay for such samples has been developed but it is too early to discuss the initial findings. One final area that the Biochemistry Unit continues to be involved with, is the development of new analytic methodology. For example, in studying the influence of Streptozotocin on the incorporation of [^{3}H]-thymidine into DNA, in studies of the alkylation of DNA by Streptozotocin and in other studies involving the possible use of [^{14}C]-formate as a marker of protein synthesis, a number of chromatographic, fluorometric, radiochemical and spectrophotometric methods have been devised.

B. Comparative Pathology Unit

The primary research efforts of this group have been in the areas of elucidating pathologic effects of antineoplastic agents and in defining animal models of human diseases. The studies of pathogenesis of adriamycin-induced cardiomyopathy in rabbits have made available to the Drug Development Program a useful animal model for assessment of the relative cardiotoxicity of adriamycin analogues. The Comparative Pathology Unit has provided the leadership in the development of this rabbit model. For the first time a small animal model is available for comparative toxicologic testing of a series of analogues. It is hoped that by such testing of analogues of adriamycin that those which have similar or higher cardiotoxic potential than the parent drug will not be employed in patients. Some adriamycin-analogue-testing may have to be done on a smaller animal model because of very limited drug quantities. Therefore, either independently or in collaboration with others, this Unit is studying the possibility of using the rat and/or the mouse as an additional model. To date however, of all the species tested only the rabbit and the monkey appear to develop a syndrome of congestive heart failure, the major dose-limiting toxicity in patients receiving adriamycin. The finding of calcium accumulation in myocardia of adriamycin-treated rabbits suggests that myocardial membrane permeability is altered. In another important related area, a rabbit tumor, i.e. VX_2 carcinoma, has been utilized to assess simultaneously the toxicity and therapeutic efficacy of adriamycin. This model may be of potential value in a later phase of testing adriamycin analogues. Major efforts also have continued in the general area of mineral and skeletal metabolism. Again the VX_2 carcinoma has provided an excellent prototype since rabbits develop hypercalcemia associated with the malignancy. In another target-organ-program, this Unit is investigating the pathologic effects of antineoplastic platinum analogues on the kidney. Since early results using the mouse were inconsistent, the rat is being tested as an alternative model. Preliminary results indicate the rat to be superior in assessing this toxicity. As mentioned in last year's report, a major discovery by this Unit was the identification and characterization of a genetic lysozyme deficiency in rabbits. Now that a number of animals are available for breeding, investigations are underway to better define the pathophysiologic role of lysozyme. The systematic evaluation of clinical and autopsy records from cancer patients who have received anticancer therapy in the Clinical Center continues. To date, five hundred autopsy records have been computerized and are available in a format suitable for querying. For example, it is now relatively simple to conduct a computer scan of these autopsy records with a question regarding the possible incidence of antineoplastic therapy-related lesions associated with a specific modality (i.e. x-ray, surgery, chemotherapy, or any combination thereof).

C. Drug Interaction Unit

The major reason for the existence of this Unit is because of the recently recognized fact that patients often receive several drugs simultaneously, and the combination may result in an undesirable effect due to what is generally categorized as "a drug-drug interaction". This is particularly a problem in patients with neoplastic disease, since they frequently receive a multitude of drugs which may alter the absorption, binding, distribution, excretion, metabolism or pharmacokinetics of the anticancer agent. Hence, methodology in this Unit has been developed and applied in a number of areas using a variety of antineoplastic agents. The principal activities of this Unit are in the areas of drug metabolism and drug transport. Such activities are of importance not only in defining which parameters lessen drug toxicity, e.g. microsomal induction, but also in understanding factors increasing toxicity. These studies recently have achieved more importance because of the evidence gathered over the past few years that drug biotransformation, leading to irreversible binding to tissue molecules, is a major parameter associated with toxicity. More and more citations in the recent literature are offering evidence that relatively inactive drugs can be activated *in vivo* to proximate toxicants. In one very important basic study conducted by this group in the past year, the actual effect of enzyme induction on over-all lethality was assessed in mice pretreated with phenobarbital. Simultaneous assessment was obtained in these animals of enzyme induction and its effect on the acute lethality produced by certain commonly employed drug metabolism substrates. This study is remarkable, primarily because it had not been done despite numerous literature citations of what the expected trends would be. Hence, in the mouse, it was shown that the enzyme-inducing process was not important in the over-all lethality for the majority of drugs tested. On the other hand, the study was important from the point of view that it did show that there was a significant increase in the LD_{50} for the analgesic narcotic ethylmorphine. A major emphasis of this research group has continued in the area of species differences in drug metabolism. These studies are important since any drug development program is always seeking to discover the animal which metabolizes drugs in a manner most similar to man. Despite several years of lip service to this important concept, it turns out that very few investigative groups have used similar methodology in assessing the ability of a wide variety of species to metabolize drugs by both hepatic and extrahepatic organs. Hence, in the past year, this group has compared on a very equitable basis the *in vitro* drug metabolizing capability of pulmonary, renal and hepatic enzymes in rats, guinea pigs, mice, rabbits and hamsters. In extending this species study to other areas, studies were initiated on Rhesus monkey, Squirrel monkey, Tupaia (a primative primate species), minipig and rat to assure comparability with the previous segment of this work.

Part of the reason for undertaking these studies is to get some information on which species might most closely resemble, not only man, but also the Rhesus monkey. This latter species is becoming increasingly scarce and it is felt necessary to try to define a species as a possible replacement if this shortage continues. At the present, there is no information upon which such a decision can be made. This Unit has also been active in studying the disposition of cis-diamminedichloro platinum in dogs. The first phase of these studies is nearing completion and it has been found that a combination of disposition and pharmacokinetic studies provides a method for the rapid accural of information on such compounds. For example, in dogs the plasma levels decayed with an initial half-time of one hour and a final half-time of about 42 hours. Remarkable changes (i.e. thirty fold) occurred in the clearance of this compound and appear to coincide with similar changes which were observed in the *in vitro* binding of this platinum analogue to plasma protein. Such evidence is suggestive of a spontaneous process, perhaps involving exchanges of the amino and/or chloride portions of the molecule. Similar distribution studies conducted in the dogfish shark suggest that exchanges involving the chlorine moiety were more important because in this aquatic species, which has about two to three times greater plasma chloride ion concentration than does the dog, such an exchange of chloride from the drug compared with the plasma was inhibited about three fold.

Early results of distribution studies in the dog have shown that the liver and kidney particularly accumulate the drug. Further studies are planned to assess the simultaneous pharmacokinetics and toxicity of this agent because of the profound differences seen in the clearance. If for example, it is discovered that the major change in the clearance is due to the renal toxicity then the fact that this shows up after a single dose is of major importance in the planned repeated-dose therapy in patients.

Studies have been conducted on drug transport, in general, and in particular biliary and renal transport. The primary model for these studies over the past few years has been the dogfish shark. The advantage of this species seems to be that it is capable of accomplishing transport and metabolic changes "in slow motion". This has proven to be of particular advantage in the study of antineoplastic drugs which are metabolized and transported sometimes at rates too rapid to accurately measure. Studies on biliary transport are important in this field, first in understanding which properties of drugs are important for biliary transport. It is hoped that such studies will provide a basis for selecting drugs which follow the biliary route as a major route of excretion and thus might be suitably employed in the chemotherapy of gastrointestinal cancers. These studies are desperately needed because of our relatively poor record of ameliorating cancers in this organ system. Study of hepatic excretion also is important to the pharmacologist in characterizing the metabolism and clearance of a drug by the body. For example, a drug which undergoes enterohepatic circulation can remain in the body for considerably longer times then might otherwise be expected. When this does occur, one must alter dose-scheduling and anticipate higher levels of toxicity. In a third area of study of the influence of drugs on biliary flow, it is of interest to develop methodology to accelerate these processes when it is desired to rapidly remove a drug *via* this mechanism. For example, it has been shown that for many drugs there is an accelerated biliary transport after prolonged treatment with the sedative phenobarbital.

D. Collaborative Efforts with Other NCI Units

Over the past year, the Laboratory of Toxicology has continued in its efforts to increase the levels of interaction with other segments of the Drug Development Linear Array. Efforts to improve the awareness level of mutual problems in the preclinical and clinical areas continue to be fruitful. For more than one year, this Laboratory has participated in Phase I, II and III Meetings and this attendance has been rewarded with many mutually beneficial exchanges of information. These exchanges of information have been translated wherever necessary, by follow-up laboratory efforts either inhouse or via the contract mechanism. Members of the Prime Contractor's Staff have also attended these clinical meetings and, in fact, have extended the level of interaction by site-visiting many of the Phase I clinicians in order to see first hand the actual work situation for these individuals. Some members of the Laboratory of Toxicology have continued their collaboration with the Membrane Transport Section, OADET, DCT, in the application of the dogfish shark as a model to study blood-brain-barrier phenomena of antineoplastic agents and model compounds. These studies have been summarized elsewhere. In another collaborative effort involving the Office of the Director, DCT, and Medical Oncology, we have just begun an approach to studying the potential use of intraperitoneal drug administration in attenuating the intra-abdominal complications associated with ovarian cancer. The current phase of this study would be classified as being in a "library" mode because, as so often turns out in biomedical science, many of the basics are not well worked out. The second phase is envisioned as a "laboratory" mode involving the use of animals in studying the properties of absorption and transport of drugs across peritoneal membranes. The major goal of this work will be to attempt to achieve high intra-tumoral levels of antineoplastic agents while minimizing systemic toxicity. The Laboratory of Toxicology has the requisite combination of skills to approach this problem since it will primarily involve the collaboration of morphologic pathologists, pharmacologists, and toxicologists. Personnel from the Medical Oncology Group will continue to provide advice as to the clinical relevancy of the proposed research. If any likely treatment-regimens are developed in the animal studies, the Medical Oncology Group will implement them in patients.

E. Collaborative Efforts with Other NIH Units

Collaborative efforts of the Laboratory of Toxicology with a number of other NIH units have continued. The major basic efforts in these programs have been in the fields of pharmacology and toxicology. These studies stem from realizing the important obligations those of us who work with drug xenobiotics owe to our less fortunate colleagues working with other xenobiotics. The over-all predictive value in extrapolating effects from animals to man is poor for most xenobiotics. But this is primarily due to the inadequacies of the human toxicologic data and secondarily due to the nature of animal test procedures. Precise knowledge of dose and schedule are virtually impossible to obtain in the case of environmental agents such as insecticides. Because those of us working with drugs can more precisely analyze the relationships we have uncovered between animal and human toxicity, and because we know the dose and schedule, we have an extra obligation to pass on our findings to our colleagues working with non-drug xenobiotics. The basis for this statement is that since these foreign compounds apparently obey the same general principles in regard to interactions with organisms, then the predictiveness of our work when extrapolated from animals to man, becomes a cornerstone for other workers. As was reported in last year's annual report, such studies often lead to a convergence of environmental and cancer research areas. For example, collaborative studies with the Biomedical Engineering and Instrumentation Branch, DRS, have centered around studies of the physiologic disposition of anticancer agents containing platinum, e.g. cis-diamminedichloro platinum. The project represents an interesting convergence of a problem of relevance to both the cancer and environmental areas. Several federal agencies have been anticipating the potential problem of platinum wastes in the biosphere since this metal is used in catalytic exhaust systems of motor vehicles. Since it is estimated that such catalysts would not last more than five years, the work this research team is doing to solve an antineoplastic drug problem, also is important in establishing the disposition of platinum in animals. Furthermore, by using highly sensitive non-isotopic methods, they have made available to this biomedical community, methods applicable to humans. Once the disposition of this agent in animals is understood, it will be rather facile to extrapolate such studies to what might happen if platinum wastes are absorbed by humans. Other work done in collaboration with NIEHS has centered around studying marine pharmacology at several levels. While the bases for these studies have been spelled out elsewhere, it should be mentioned here that this work has widespread relevance, since the oceans are now considered to be the "ultimate sink" for all xenobiotics. Hence those who are best qualified to do so should characterize the effects of xenobiotics on marine species and the effects of such species on xenobiotics. Such baseline information is of importance to all mankind since it will raise the awareness level that a resource like the ocean cannot be polluted indefinitely by drugs and other xenobiotics.

III. Contract Programs

A. The Prime Contract

We have now completed one year of operation under the Prime Contract mechanism. The technical aspects of this program are working out exceedingly well. One problem area which was experienced during the past year, i.e. in the contract area, was largely due to personnel turnover. It would appear that these problems are well underway to resolution. During the first contract year, the Prime awarded two subcontracts and is in the process of awarding two to three additional subcontracts. The major goal of the Prime Contract is to continue the protocol toxicity studies in large animals and it has served its goal very well by completing the first assigned protocol tasks within eight to nine months. Not only has the Prime provided the Division with a mechanism for the rapid completion of such studies, 20-150% faster, it has done so with some cost savings. During the first contract year only about one-half the projected number of protocol drugs were available to the Prime, but for the present contract year it would appear that the projected number of compounds will indeed be assigned. The major impact of the Prime has been that it has made available more research time for the internal staff, so that they can pioneer methodology which when adequately worked out, can then be taken over by the Prime. This is a vital role in a rapidly changing field such as is modern toxicology.

B. Present Contract Programs

Most of the contracts supported by the Laboratory of Toxicology for protocol toxicity studies were terminated as of March, 1974 and to date, two from this original group have been brought aboard under the Prime Contract mechanism. The Laboratory of Toxicology also supervises four other contracts. Two of these are in the area of drug metabolism wherein these groups (University of Oregon and Stanford Research Institute) are conducting studies primarily on the metabolism and other aspects of disposition of antineoplastic agents. The University of Colorado is under contract to study the cardiotoxicity of adriamycin and this contract has been very fruitful in aiding the Laboratory in developing the rabbit model as mentioned earlier in this report. The fourth contract is with Dow Chemical Company and is involved with reproductive and teratogenic toxicity of antineoplastic agents. This resource was developed in answer to FDA's request to provide such information for antineoplastic agents at the NDA phase. This has been a very productive contract in that in the past three years, they have completed full studies for eight agents and this information has already been used or is about to be used to support several NDA applications to FDA.

Serial No. Z01 CM 03518-07 LT
1. Laboratory of Toxicology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: L-Asparagine: Synthesis, Biological Role and Hydrolysis
Streptozotocin: Alkylation of Nucleic Acids

Previous Serial Number: NCI-3518

Principal Investigator: David A. Cooney

Other Investigators: Harry Milman, Ritchard Cable, Benjamin Taylor

Cooperating Units: Philip S. Schein, Georgetown University, Washington, D.C.
Isadore Wodinsky, Arthur D. Little, Inc., Boston, MA
Robert Pittillo, Southern Research Institute, Birmingham, AL
David Houchens, National Cancer Institute, NIH, Bethesda, MD
Howard Bruckner, Mt. Sinai School of Medicine, New York, NY

Man Years:

Total:	5
Professional:	4
Others:	1

Project Description:

Objectives:

To examine the pharmacological properties of oncolytic enzymes and to examine the mechanism of the toxicity of select nitrosoureas.

Major Findings:

1. Interiorization of L-Asparaginase in Syngeneic Erythrocytes

Pure L-asparaginase from *Escherichia coli* (EC 3.5.1.1) and crude L-asparagine synthetase from Leukemia 5178Y/AR (EC 6.3.5.4) have been forced into the cytoplasm of mouse erythrocytes whose pore-size was transitorily enlarged by exposure to cold hypotonic solutions containing these enzymes along with homologous murine hemoglobin. Chromium labelling of such enzyme-laden erythrocytes - or 'zymocytes' - reveals that their half-life *in vivo* is shorter than control cells. Antibodies in the serum of mice given intravenous injections of L-asparaginase appeared whether or not the enzyme was interiorized before its administration. However, the duration of eradication of L-asparagine from plasma was roughly twice as long in subjects receiving 'zymocytes' containing L-asparaginase as in controls given equivalent doses of free enzyme. The therapeutic activity of such cells is currently being explored.

2. Studies with L-Asparagine Synthetase

a. Methodologic Considerations. Four variations of a radiometric micromethod for the measurement of L-asparagine synthetase activity have been compared systematically. Each utilizes bacterial L-asparaginase to hydrolyze newly synthesized L-[4-^{14}C]asparagine, and L-glutamic acid oxaloacetate transaminase to convert the L-aspartic acid so generated to oxaloacetic acid - a compound readily β-decarboxylated by zinc ions. The advantages of each method in a particular experimental setting have been examined from the standpoint of speed and convenience. A vigorous evaluation of the reliability and applicability of these techniques has also been conducted. Lastly, the modifications have been compared with all known and published techniques for measuring L-asparagine synthetase activity and concluded to be superior from several standpoints.

b. The Purification of L-Asparagine Synthetase from Mouse Pancreas. Resistance to L-asparaginase correlates with the presence of elevated activity of L-asparagine synthetase in the tumor cell. To render such cells sensitive once again to L-asparaginase therapy it would, therefore, be desirable to interrupt the synthesis of L-asparagine. Towards this goal, our laboratory has been searching for effective inhibitors of L-asparagine synthetase of tumoral origin. It is noteworthy that normal pancreas also contains a vigorous L-asparagine synthetase whose reaction-mechanism appears to be analogous to its tumoral counterpart. Therefore, drugs used *in vivo* as inhibitors of L-asparagine synthetase of tumoral origin would, conceivably, also inhibit the pancreatic enzyme. From a toxicological standpoint it was felt to be important to attempt to purify L-asparagine synthetase from pancreas and compare it with its tumoral counterpart in the hope of finding significant differences which would ultimately be useful in specific drug therapy. With this in mind, we have undertaken the following procedures in the purification of the enzyme from mouse pancreas:

(1) Homogenization of mouse pancreas in 0.01 M Tris formate buffer, pH 7.8, containing 1 mM dithiothreitol, 0.5 mM Na_2-EDTA, 1 mM L-glutamine, 1 mM Mg-formate, and 1 mM ATP.

(2) Addition of 0.001 M diisopropylfluorophosphate (DFP) to the crude homogenate. Inasmuch as the pancreas contains a large number of proteolytic enzymes which are known to digest L-asparagine synthetase and inasmuch as many of the proteolytic pancreatic enzymes have serine in their active sites, DFP - a serine alkylating agent - has been added at this stage.

(3) Centrifugation at 105,000 x g (20 minutes). This step, which serves to sediment the insoluble proteins yields 4-6 fold purification, with full recovery of activity.

(4) Subjection of the 105,000 x g supernatant to DEAE-A25 (formate form) ion-exchange chromatography. An ammonium formate gradient (0.05-1.0 M) is used in the presence of 1 mM L-glutamine, dithiothreitol, and ATP-$MgCl_2$ and 0.5 mM EDTA. This step yields an additional 30 fold purification with recoveries in the neighborhood of 75-85%.

(5) Ammonium Sulfate Precipitation. The active fractions from Step 4 are pooled and brought to 30% saturation with solid ammonium sulfate. The precipitate is discarded and the supernatant brought to 50% saturation with solid ammonium sulfate. The precipitate is collected and taken up in a small amount of homogenization buffer.

(6) Sephadex G-200 Filtration. The 30-50% ammonium sulfate fraction is loaded on a Sephadex G-200 column and eluted with 0.1 M Tris formate buffer, pH 7.8 containing 1 mM ATP-$MgCl_2$, L-glutamine, and dithiothreitol and 0.5 mM EDTA. A 5 fold purification is achieved in this step with full recovery.

(7) Ammonium Sulfate Precipitation. The active fractions of Step 6 are precipitated with ammonium sulfate to 30-50% saturation or their volume is decreased by Amicon ultrafiltration.

(8) Sucrose Gradient. The fraction from Step 7 is loaded on a sucrose gradient (5-20%) and centrifuged at 200,000 x g at 4° for 22 hours in an ultracentrifuge. This step yields an 11 fold purification with 100% recovery.

(9) Preparative Gel Electrophoresis. This method utilizes 5% acrylamide polymerized in a Canalco preparative unit. A 1.5 fold purification is achieved. Utilizing the methods outlined above, about 1,000 fold purification over the crude homogenate is produced; the final preparation is still heterogeneous so that additional steps ultimately will be needed to achieve purification to homogeneity.

c. Inhibition of L-Asparagine Synthetase

i. The Effect of Miscellaneous Metallic Ions on the Activity of L-Asparagine Synthetase. Preliminary studies have shown that L-asparagine synthetase of L5178Y/AR and mouse pancreas are affected by various metal ions. A closer investigation revealed that salts of copper, zinc, cadmium, and cobalt as well as calcium and manganese inhibit these enzymes, in vitro, at a concentration of 1 mM, by 70-99%. In vivo, however, even at high doses (100 mg/kg) these agents were not found to inhibit significantly either of these enzymes (15-30% inhibition). Increasing the magnesium ion concentration (magnesium being the ordinary metal co-factor in the synthesis of L-asparagine), in vitro, failed to prevent or reverse established inhibition. In the case of copper and cadmium, dithiothreitol at 5 mM was able to prevent inhibition, in vitro. Moreover, EDTA at 2 mM reversed the inhibition exerted by zinc, cadmium and manganese by 45-93%. None of the divalent cations studied were able to substitute appreciably for magnesium at concentrations up to 10 mM. It appears that certain of these ions, in body fluids, notably calcium and zinc, may have a regulatory effect on the activity of this important biosynthetic enzyme.

ii. Endogenous Inhibitors. An investigation has been carried out on certain endogenous inhibitors of L-asparagine synthetase. These can be defined as miscellaneous compounds occurring in the body fluids of mammals and found to be capable of significant inhibition of L-asparagine synthetase. The nature of these inhibitors was deduced by adding counteragents of known specificity and capable of nullifying their influence to organ extracts being assayed. Thus, EDTA or EGTA were used to remove calcium ions, protamine sulfate of lysozyme to remove heparin and heparinoids, ammonium sulfate or potassium sulfate to inhibit contaminating endogenous L-asparaginase, dilute trypsin to digest endogenous proteinacious inhibitors (L-asparagine synthetase being shielded from proteolytic attack by the presence of its substrates) and dithiothreitol to remove traces of thiol-reactive metals or endogenous sulfhydryl ligands. These agents individually achieved a 130 to 150% augmentation of the activity of the synthetase in extracts of leukemia 5178Y/AR and normal mouse pancreas; used in concert, they permitted a 2 to 3 fold augmentation of the apparent activity of L-asparagine synthetase in extracts from both sources. However, despite the addition of these counter-agents to hepatic extracts whose content of this enzyme was being assessed, only marginal L-asparagine synthetase was demonstrable in liver. Thus, although perfusion experiments have demonstrated conclusively that liver is a chemostat for L-asparagine; the enzymic basis for this regulation is unclear.

d. New Inhibitors. N-Methyl maleimide, a potent sulfhydryl reagent, has been found to interfere selectively with the utilization of L-glutamine, as opposed to ammonia (an alternate substrate), by several L-glutamine amidotransferases. Thus, a 1 mM concentration of this agent inhibits the synthesis of L-asparagine, NAD and phosphoribosylamine from L-glutamine and an appropriate acceptor, by 50% or greater *in vitro*. Dialysis fails to reverse established inhibition, but L-glutamine, in molar excess, can prevent the effect. A kinetic analysis of inhibition exerted by N-methyl maleimide versus L-asparagine synthetase from leukemia 5178Y/AR (resistant to L-asparaginase) yielded patterns which were apparently competitive in type when L-glutamine was the nitrogen donor; activation was seen with ammonia as substrate. An examination of the antimicrobial action of N-methyl maleimide towards bacteria or yeasts with known patterns of resistance to the prototypical analogs of L-glutamine, azaserine (O-diazoacetyl-L-serine), DON (Norleucine, 6-diazo-5-oxo, L-), DONV (Norvaline, 5-diazo-4-oxo, L-), and NSC-163,501 (L-[αS,5S]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) indicated that organisms sensitive to DON and azaserine were ordinarily sensitive to N-methyl maleimide; resistance, however, was seldom collateral in this group of agents, and complicated relationships emerged from this study. *In vivo*, N-methyl maleimide failed to produce an expansion of the pool size of L-glutamine in tumor, liver and pancreas. In this respect, the agent differs from DON and NSC-163,501; moreover, unlike the latter two drugs, N-methyl maleimide was devoid of oncolytic activity against leukemias 1210, 5178Y/AS or 5178Y/AR.

e. L-Asparagine Synthetase in Serum as an Index of Neoplasia. L-Asparagine synthetase was found to appear in serum 7 days after the subcutaneous implantation of 1 x 10^5 cells of leukemia 5178Y/AR (resistant to L-asparaginase), and increased in concentration as the tumor grew and metastasized. The principal source of the enzyme is the primary tumor. The enzyme was found to be removed from the serum with a half-life of 1 hour whether or not the LDH-elevating virus was present. After intracranial inoculation, the rate of leakage of the enzyme was more pronounced than when the subcutaneous, intramuscular, or intraperitoneal routes were used. CCNU (Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-, NSC-79037), a nitrosourea effective in the palliation of L5178Y/AR, temporarily halted the influx of enzyme into the bloodstream, as did surgical excision of the subcutaneous tumor nodules. Treatment of mice with L-asparaginase within 24 hours of inoculation of the tumor markedly augmented both tumor-growth and the rate of penetration of L-asparagine synthetase into the circulation. Several other L-asparaginase-resistant tumors also were found to spill L-asparagine synthetase into the serum, but the correlation between this phenomenon and the specific activity of the enzyme in homogenates of the tumor was imperfect.

f. L-Asparagine Synthetase: A Marker for Metastasis of Murine Tumors. Leukemia 5178Y/AR (resistant to L-asparaginase) contains L-asparagine synthetase (EC 6.3.5.4) at high specific activity in its cytoplasm (1.5 fmoles/hr/cell). As this neoplasm grows in the inguinal tissue of BDF_1 mice, it metastasizes widely. L-Asparagine synthetase has been found to increase in concentration in liver, spleen, kidney and other tissues as a concomitant of such metastasis. Treatment with L-asparaginase on the day prior to, or following inoculation of tumor is known to augment metastasis to lung (Fidler, Nature, 229, 564, 1971). In the present studies, pretreatment with L-asparaginase also augmented the concentration of L-asparagine synthetase in the liver of tumor-bearing mice, especially during the early stages of tumor growth. When the concentration of viable tumor cells in liver, as determined by bioassay, was compared with the level of hepatic tumoral L-asparagine synthetase, an acceptable correlation was observed. However, histopathologic assessment of random liver sections proved to be inferior to both the bioanalytical and enzymologic methods as an index of metastasis.

g. Measurement of L-Asparagine Synthetase in Clinical Serum Specimens. In collaboration with Drs. Howard Bruckner and Philip Schein, a survey is being conducted for levels of L-asparagine synthetase in the serum of a variety of patients with malignancy. To date, 50 serum samples from 50 subjects have been analyzed by radiometric methodology capable of reliably detecting the synthesis of as little as 4.09 pmoles of L-asparagine. No evidence for activity of the enzyme has been found in these cases. However, it should be stressed that no attempts have thus far been made to reverse endogenous inhibition of L-asparagine synthetase with counteragents.

h. Studies with New Oncolytic Enzymes. In collaboration with Dr. John Douros, a *Clostridium* species rich in L-methioninase is being grown on a large scale at the Frederick Cancer Research Center. It is planned to isolate the enzyme according to the methodology of Dr. Willi Kreis of Sloan Kettering and to test its effect first on tissue culture and then against intraperitoneal transplantable tumors. During the past year, two new purified batches of L-phenylalanine ammonia lyase were sent to Dr. Florence White and the Laboratory of Toxicology from Dr. C. W. Abel of the University of Texas, Galveston for simultaneous antitumor and toxicologic testing in mice. Both lots were rapidly lethal to the recipients at all doses. A new chromatographic step has been introduced into the purification system which successfully removes the toxin responsible for the lethality observed. As soon as sufficient toxin-free enzyme has been purified, studies with this new oncolytic preparation will be resumed.

3. The Effects of Streptozotocin (Glucopyranose, 2-deoxy-2-(3-methylnitrosoureido)-D-) (NSC-85998) on Mammalian Nucleic Acids. Streptozotocin is a nitrosourea antibiotic with significant oncolytic activity. Its glucosamine function has been postulated as the basis for its affinity for pancreatic islet beta cells. In fact, Streptozotocin has been shown to destroy normal beta cells of the pancreas, resulting in a diabetic syndrome in many species but seldom in man. Nicotinamide effectively blocks this action. Finally,

Streptozotocin administered to Holtzman rats pretreated with nicotinamide has been shown to cause nesidioblastomas (islet cell tumors of the pancreas) within 300 to 500 days.

a. The Influence of Streptozotocin on Incorporation of [^{3}H]-Thymidine into DNA. In Vivo. Mice bearing L1210 tumors sensitive to streptozotocin (NSC-85998) were given a single 50 mg/kg dose of the drug. A parallel group of mice bearing the Lewis Lung tumor (reported to be resistant to streptozotocin) were included for purposes of comparison. Controls for both groups received the vehicle alone (saline citrate, pH 4). Five, 23 and 47 hours after injection of streptozotocin, mice from all groups were injected with [^{3}H]-thymidine (33 μCi/mouse); one hour later the mice were sacrificed and the tumors, livers and spleens removed. The DNA from these organs was isolated from homogenates using a modified Marmur method followed by treatment with ribonuclease; the purity of the DNA samples was gauged by the absence of a uracil peak on chromatography. Inasmuch as samples were free of uracil, it was concluded that RNA had been essentially removed. For estimation of DNA, the diphenylamine method of Burton was used. The data were summarized as pmoles of [^{3}H] TdR/μg DNA. For the most part there was no striking difference between the control and experimental animals for each group except the values for uptake and incorporation in the livers of mice bearing L1210 were appreciably higher than the values for Lewis Lung livers. Experiments also were begun using an L1210 tumor-line rendered resistant to streptozotocin. Experiments are underway to determine levels of alkylation and to measure the half-life of the 7-methyl guanine species in the sensitive vs the resistant variants of this tumor line.

b. Studies with Streptozotocin. In Vitro. L1210 cells grown in suspension cultures at levels of 1.5 x 10^6 cells/ml were used in a series of experiments to compare the alkylating effects of streptozotocin with other known alkylating agents. Tissue cultures of 1000 ml or more were sub-divided into several smaller cultures for parallel experiments. Doses of nitrogen mustard (HN2), NSC-762 and of N,N-diethyl-2-chloroethylamino, (HN1A), NSC-2059 were compared with streptozotocin at levels of 10, 1 and 0.1 mM. Untreated controls also were run and all cultures were pulsed with equivalent amounts of [^{3}H]-thymidine. In all cases the incorporation of thymidine was inhibited in comparison with controls; however, the inhibition (or cell destruction) with HN2 was much greater (10X) than with either HN1A or streptozotocin. It was of interest that the gross effects of thymidine uptake and incorporation for both HN1A and streptozotocin were quite similar, leading to the working hypothesis that dissimilar alkylating agents may have a common mechanism of attack on DNA. Although no definitive quantitative information is yet available, 7-methyl guanine and other alkylation products have been detected using very sensitive UV methods and fluorimetry.

c. Sample Separation and Quantitation. DNA samples have been hydrolyzed using varying concentrations of HCl, $HClO_4$ (60 or 70%) and HCOOH with generally the same results. Hydrolysis of samples for 1 hour at 95° leads to almost complete hydrolysis of the DNA - to the free bases with $HClO_4$ and to varying degrees of completeness with the other acids. After exposure of base standards, including O-6-methyl guanine (which was kindly supplied by Dr. Lawley of the Chester Beatty Institute, London, England) to the conditions used for the hydrolysis of DNA, it was found that acid concentrations greater than 1N converted, 0-6-methyl guanine to guanine. With dilutions of HCl (0.1 and 0.01 N), the decomposition of the 0-6 species was decidedly reduced. Dilute acid treatments of samples of 7-methyl guanine had little or no adverse affect on this species. Electrophoresis, paper chromatography and column chromatography have been employed for DNA analysis. With electrophoresis of standard samples, there was no clean-cut separation of methylated guanines. Paper chromatography of depurinated [^{14}C]-streptozotocin-labelled DNA yielded a large 7-methyl guanine peak (about 60% of the radioactivity) and a smaller peak with a R_f approximating that of 0-6-methyl guanine. With column chromatography, qualitative information as to the presence of such minor components as 0-6-methyl guanine has been attempted; however, no clear-cut evidence for the presence of this species presently exists from experimental samples.

d. Production of Pancreatic Islet Cell Tumor. Fifty Holtzman rats have been treated with Streptozotocin and nicotinamide at doses found in the past to be productive of islet cell tumors. The subjects are now 8 months post-treatment and all are still normoglycemic. It is anticipated from prior studies that the neoplasms will begin to influence the blood sugar at about 12 months post-treatment.

4. New Analytical Methodology. Because of its rapid incorporation into guanine and adenine residues in the *de novo* pathway of purine biosynthesis, [^{14}C]formate has found widespread use as an indicator of nucleic acid metabolism *in vivo*. In like manner, L-[^{14}C]valine has been used extensively for assessment of the rate of protein biosynthesis. However, unlike L-valine, formic acid has a plentitude of metabolic fates. One of these is incorporation into the methyl group of L-methionine. Inasmuch as such newly synthesized L-[^{14}C]methionine equilibrates with the general L-methionine pool for incorporation into proteins, it seemed to us that [^{14}C]formate could be made to function as a useful indicator of protein biosynthesis. In this way a single isotopic precursor would provide information on two vital macromolecular biosynthetic processes. For quantitation of the specific radioactivity of L-[^{14}C] methionine in protein, the residues remaining from hot salt extractions of organ homogenates were dissolved in 500 µl of 20% formic acid. Then, 500 µl of 1% cyanogen bromide in toluene was added and the mixtures incubated at room temperature for 10 hours. During this time L-methionine is degraded by cyanogen bromide to homoserine lactone and methyl thiocyanate. Methyl thiocyanate, the product, being sparingly soluble in water but extensively soluble in organic solvents, enters and is retained by the organic phase. At the term of the reaction a small aliquot (usually 50 µl) of the toluene supernatant is taken for the estimation of radioactivity by scintillation spectrometry, and another aliquot for the measurement of total methyl thiocyanate by gas chromatography. The method is facile, and apparently specific - provided that an acid pH is used for the decomposition of L-methionine.

Significance to Biomedical Research and the Program of the Institute:

To date, the number of enzymes that have oncolytic activity is small, and confined mainly to hydrolases copable of eradicating essential or quasi-essential amino acids. Nevertheless, it appears warranted to generalize that such enzymes are powerful, and on occasion, selective tools for attacking cancer. The present series of studies seeks to uncover small molecules which will broaden the therapeutic scope of such exogenous enzymes by inhibiting the induction or function of endogenous synthetases responsible for resistance. Studies on the alkylation of DNA by streptozotocin suggest a mechanism for the oncogenicity of this drug.

Proposed Course:

Systematic screening for inhibitors of L-asparagine synthetase will continue. Studies on new oncolytic enzymes will continue. It is hoped that non-denatured extracts of cultures and plants will begin to be examined in the anticipation of the discovery of macromolecular oncolytics in these sources.

Keyword Descriptors:

L-Asparagine
Enzyme chemotherapy
Streptozotocin
Nucleic-acid-alkylation

Publications:

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1. Laboratory of Toxicology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Pathology of Drug-Induced Alterations in Animals and Man
Subtitle: The Characterization of Animal Models for Human Disease

Previous Serial No. Same

Principal Investigator: David M. Young

Other Investigators: Ritchard G. Cable, Jerrold M. Ward, Harry M. Olson, Reginald L. Reagan, David P. Houchens, Louis B. Thomas, Arnold W. Pratt, Stephen H. Rosenoff, and M. Collins

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Man Years:

Total:	5
Professional:	3 1/2
Other:	1 1/2

Objectives:

1. To define the clinicopathologic effects of adriamycin (ADR) in rabbits, mice and rats and characterize an animal model for:
   a. analogue testing
   b. antidotal testing
   c. antitumor and cardiotoxicity testing
2. To characterize the physicochemical and morphologic alterations in chondro-osseous tissues induced by long-term ADR administration.
3. To characterize the transplantable VX_2 carcinoma in rabbits as a model for:
   a. hypercalcemia of malignancy
   b. testing of oncolytic and cardiotoxic activity of ADR and its analogues
4. To evaluate morphologic and biochemical alterations of skeletal muscle in rabbits with ADR-induced cardiomyopathy.
5. To define an animal model for experimental chondro-osseous neoplasia for:
   a. investigation of biologic behavior and pathogenesis

b. testing antineoplastic agents for activity against chondro- and osteogenic sarcomas.

6. To evaluate the adverse effects of ADR on calcium homeostasis and to correlate these findings with the known calcium accumulation in affected myocardia.
7. To determine the role of lysozyme in chondro-osteogenesis under physiologic and pathologic conditions.
8. To evaluate the rat as a model for determining nephrotoxic effects of antineoplastic agents.
9. To characterize the hematopoietic and chondrogenic lesions in Alaskan Malamutes with hereditary chondrodysplasia as a model for dwarfism in humans.
10. To systematically evaluate the clinical and autopsy records for evidence of adverse effects of antineoplastic agents in cancer patients on a computerized basis and correlate these findings with those recorded in test animals.
11. To provide advice, consultation and support in the area of pathologic evaluation for senior investigators within the DCT.

Major Findings:

1. The Clinicopathologic Effects of ADR in Rabbits, Rats, and Mice. Recent reports of the toxic effects of ADR in animals and humans indicate that the tissues of the following eight organ systems are adversely affected: hematopoietic, lymphoreticular, digestive, reproductive, cardiovascular, urinary, skeletal and integumentary. Myelosuppression, digestive disturbances, stomatitis and alopecia are the most frequently occurring adverse effects in patients receiving ADR. It has been well accepted that the cardiotoxicity of ADR is the major total dose limiting toxic manifestation in patients. A number of animal models have been characterized to various degrees as indicators of ADR-induced cardiotoxicity. An animal model would be valuable in testing analogues of ADR for cardiotoxicity and for testing antidotal procedures. To date, only the rabbit and monkey have been reported to develop a dose-related cardiomyopathy with congestive heart failure. Smaller laboratory animals (i.e. mouse and rat) offer distinct advantages as models with respect to size, temperament, handling, care and housing required for testing large numbers of drugs. Therefore, the current investigations are designed to determine if ADR will induce a significant cardiotoxic syndrome in the rat and/or mouse. Preliminary evidence suggests that the mouse develops a cardiomyopathy after single as well as multiple doses of ADR. The morphologic changes appear to be similar, but to a lesser degree in severity, to those described in the rabbit and humans. The structural alterations correlate well with the findings of decreased myocardial DNA production and increased calcium accumulation in heart muscle. These findings are being compared with those reported in the rabbit and with those under current investigation in the rat.

2. Physicochemical and Morphologic Alterations in Chondro-Osseous Tissues Induced by ADR. Long-term, low dose administration of ADR to young growing rabbits resulted in morphologic changes which were most pronounced at epiphyseal and metaphyseal areas of long bones. Results of in vitro studies indicate that ADR binds readily to nondemineralized, fresh cortical bone powder.

The findings in ADR treated rabbits of decreased bone density, histopathologic alterations, and a paucity of osteogenic cells are interpreted as retardation of bone maturation. Further investigations are in progress to better define the interaction of ADR and chondro-osseous tissues.

3. Clinicopathologic Evaluation of the Transplantable VX_2 Carcinoma in the Rabbit. The intramuscular inoculation of 1 ml of VX_2 carcinoma cells from the laboratory tumor bank into weanling rabbits results in rapid tumor growth within a period of 10-14 days. A significant hypercalcemia and hypophosphatemia develops at 2 weeks post-inoculation and persists until death of the animals as a result of pulmonary metastases. Preliminary evidence suggests that the neoplasm produces a chemical mediator that stimulates increased bone resorption. In addition to investigating the VX_2 carcinoma as a model for hypercalcemia of malignancy, studies were designed to investigate the feasibility of utilizing this model to detect therapeutic efficacy and cardiotoxic activity of ADR and related analogues. The findings of this study indicate that treatment of the VX_2 carcinoma in rabbits with ADR results in (1) a significant increase in median survival time, (2) a diminution of disseminated metastases and (3) development of a cardiomyopathy associated with low cumulative doses. It is possible the characteristic hypercalcemia of the VX_2 carcinoma potentiates the cardiotoxic activity of ADR.

4. Evaluation of Morphologic and Biochemical Alterations of Skeletal Muscle from Rabbits Receiving ADR. Skeletal muscle toxicity has been detected in rabbits receiving total doses of ADR of 200-400 mg/M^2 at a dose schedule of 1.25 mg/kg I.V. twice a week. These changes are similar to, but significantly less severe than, the cardiac lesions produced in the same animals. The cardiac and skeletal muscle content of high energy phosphate compounds is being investigated in these cardiomyopathic rabbits. The effects of ADR on Ca-Mg ATPase systems or on the membrane ATPases have yet to be investigated.

5. Characterization of an Animal Model for Experimental Chondro-Osseous Neoplasia. Osteogenic sarcoma in humans is a highly malignant neoplasm with a poor five year survival rate in spite of rigorous therapeutic regimens. It has been reported recently that osteogenic sarcoma results in death within two years of onset in 4 out of 5 cases. No useful animal model has been reported for testing various chemotherapeutic modalities. Present investigations were undertaken to evaluate osteogenic sarcomas induced by the FBJ virus in mice. Osteogenic sarcomas developed 5 weeks post-inoculation and were readily transplantable to weanling mice, with 4 different tumor cell lines currently being maintained. Further investigations were designed to characterize and histopathologically classify these neoplasms for testing oncolytic agents.

6. Evaluation of the Adverse Effects of ADR on Calcium Homeostasis. Previous experiments involving analysis of serum from rabbits receiving multiple, long-term doses of ADR revealed a mild hypocalcemia in several rabbits. Serum obtained from rabbits receiving ADR at a dose of 3 mg/kg for 5 consecutive days revealed a significant hypocalcemia and hypomagnesemia. Further investigations were designed to study the hypocalcemic effects of ADR in a sensitive rat bioassay system. Results of assays utilizing doses of 12, 24, and 36 mg/kg of ADR revealed a significant linear dose-related hypocalcemic effect five hours after I.V. administration. Current investigations are in progress to determine the pathogenesis of this ADR-induced alteration in calcium homeostasis and its

role in the genesis of the cardiomyopathy. It is hypothesized that the mechanism of the acute hypocalcemic effect on ADR may be mediated through inhibition of active bone resorption.

7. Role of Lysozyme in Chondro-Osteogenesis under Physiologic and Pathologic Conditions. Lysozyme is a small, cationic, ubiquitous enzyme that has been reported to be elevated significantly in the urine and serum of patients with monocytic and myelomonocytic leukemia. In addition determination of serum lysozyme levels has been used to differentiate between two types of neutropenia. Recent studies indicate that lysozyme may play a significant role in host defenses against neoplasia and may be a mediator of antitumor function of macrophages. Further studies are needed to determine the physiologic role and antitumor activity of lysozyme. Recently a genetic disorder consisting of a deficiency of lysozyme has been reported in a strain of New Zealand white rabbits. It is suggested that these mutant rabbits may be useful for experiments designed to delineate the biologic role of lysozyme. Lysozyme, for which there is no known substrate in mammalian tissues, is present in a number of cells and body fluids including cartilage where it appears as a nonlysosomal enzyme. The present studies are designed to evaluate the response of lysozyme deficient rabbits to a rachitogenic diet. The findings of this study will aid in the elucidation of the role of lysozyme in chondro-osteogenesis.

8. Evaluation of the Rat as a Model for Testing the Nephrotoxicity of Antineoplastic Agents. A number of anticancer agents appear to produce renal damage in cancer patients. One of the major toxic effects of the newly utilized agent platinum, diamminedichloro-*cis*- is development of a nephropathy. Platinum analogues are being developed with the hope of reducing the nephrotoxicity while retaining the therapeutic effectiveness. Therefore, a laboratory animal sensitive to the nephrotoxic effects of the parent compound is urgently needed in order to test a number of analogues. Rats receiving platinum, diamminedichloro-*cis*- intravenously are currently under investigation. Serum chemistry, urinalysis, histopathologic evaluation and ultrastructural studies will be utilized in an attempt to characterize the toxic effects of such agents on the renal parenchyma. These results will aid in the development of a protocol for testing platinum analogues.

9. Characterization of the Hematopoietic and Chondrogenic Lesions in Alaskan Malamutes with Hereditary Chondrodysplasia. Chondrodysplasia is the most common cause of dwarfism in man and is easily distinguishable from the pathologic dwarfism of hypopituitarism or hypothyroidism. A hereditary chondrodysplasia occurs in Alaskan Malamutes in concert with a hypochromic, macrocytic anemia. The pathogenesis of this disorder of hematopoietic and osteogenic cells remains to be elucidated. Current studies are designed to characterize the disorder in Alaskan Malamutes utilizing roentgenographic, biochemical, histochemical, morphologic and physiochemical analyses of bones and cartilage. In addition, these findings will be compared with those reported in man to determine if the skeletal disorder in Malamutes is a good model for dwarfism in man. Further studies are designed to determine (1) if cartilage lysozyme levels are altered, (2) if aging dysplastic cartilage undergoes neoplastic transformation and (3) if the genetic defect may involve a pathologic change in a common progenitor mesenchymal cell that gives rise to osteogenic and hematopoietic cells.

10. Evaluation of Adverse Effects of Antineoplastic Agents in Cancer Patients. A specialized system of information storage and retrieval was designed and implemented in collaboration with the Division of Computer Research and Technology. This processing system employs natural language and the systematized nomenclature of pathology (SNOP). The major objective of the current studies is to determine the qualitative and quantitative aspects of adverse effects of antineoplastic therapy in cancer patients at the time of autopsy. Currently, clinical and autopsy records on a total of 500 cancer patients have been processed via this computerized system. The percentage of patients receiving chemotherapy, surgery, or radiotherapy was 87, 42, and 50 respectively. Only 5.4% of the patients received immunotherapy. Neoplastic diseases other than the type evident upon admission were found in 30% of the patients at autopsy. Septicemia was evident at autopsy in 51.6% of the patients. A total of 12% of the patients were in remission at the time of death. The most frequently utilized antineoplastic agents were prednisolone, vincristine, cyclophosphamide, methotrexate, cytosine arabinoside and 6-mercaptopurine. Studies are in progress to correlate specific lesions with various treatment modalities. The anticancer agent-associated lesions in patients will be compared with those reported previously in preclinical toxicologic studies in dogs and monkeys.

11. Advice, Consultation and Support in the Area of Pathologic Evaluation. Usually, in order to perform investigations of high scientific merit in the biomedical area, experimental animals are required. It is well known that spontaneous diseases develop in laboratory animals and are inherent problems that must be solved by a thorough understanding of the pathogenesis, and etiology of the condition. Spontaneously occurring diseases of laboratory animals have not been thoroughly investigated. In fact, new infectious agents are discovered each year. A cooperative study involving various investigators is necessary in order to elucidate the mechanism by which laboratory animals acquire, disseminate and are affected by these various agents. Intercurrent infectious diseases in laboratory animals is one of the major complicating factors in investigational studies. Recently, for example, athymic "nude" untreated mice in the Division of Cancer Treatment were found to have a fulminating pneumonia which may be caused by a new murine virus. Studies are in progress in collaboration with Microbiological Associates to isolate the causitive agent. Other diseases of experimental animals should be brought to the attention of veterinary pathologists.

Significance to Biomedical Research and the Program of the Institute: The need for a better understanding of the pathogenesis of anticancer drug-induced lesions in experimental and clinical studies cannot be overemphasized. The elucidation of the mechanism of ADR-induced cardiotoxicity may lead to establishment of a therapeutic or prophylactic procedure(s) to negate the cardiac failure syndrome. Ideally, oncolytic and toxicologic testing should be done with the same species. Currently, rabbits harboring the VX_2 carcinoma appear to be the best model to date for simultaneous detection of oncolytic and cardiotoxic activity of ADR and associated analogues.

The characterization of an animal model of osteogenic sarcoma would provide a new avenue for testing various modalities against a heretofore chemoresistant tumor type. Preliminary results suggest that the viral-induced osteogenic sarcoma in mice may be a good model for osteosarcoma in man. The characterization of animal models of organ specific toxicity for evaluation of analogues of parent drugs is an important aspect of toxicologic studies. Investigations are in progress to define an animal model for renal toxicity. The characterization of ADR-induced morphologic and biochemical lesions in skeletal muscles may provide for a clinically applicable test for impending cardiotoxicity. In addition, these studies will contribute to a better understanding of the basic ADR-induced cardiomyopathy.

Proposed Course:
The computerized data from clinical and autopsy records from cancer patients and results from preclinical toxicologic studies in dogs and monkeys are currently being summarized in manuscript form. Future studies involving tabulation and retrieval of computerized data will be handled by the prime contractor.

Intramural research activities will be continued and expanded under the following specific objectives: (a) to characterize the myopathic effects of ADR and define methods to block this toxic manifestation; (b) to better define the interactions of ADR and bone metabolism under physiologic and neoplastic conditions; (c) to characterize animal models for osteogenic sarcoma, organ specific drug-induced toxicity, and hypercalcemia of malignancy; (d) to contribute to a better understanding of the role of lysozyme in physiologic and pathologic conditions.

Keyword Descriptors:
Adriamycin
Cardiotoxicity
Osteogenic sarcoma
Lysozyme
Bone metabolism
Rickets
Chondrodysplasia
Pathology
Animal models
VX_2 carcinoma
Hypercalcemia
Hypocalcemia
Nephrotoxicity
Comparative toxicology

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Serial No. Z01 CM 06122-03 LT
1. Laboratory of Toxicology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1974 through June 30, 1975

Project Title: Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs

Previous Serial Number: NCI-6122

Principal Investigators: T. E. Gram, C. L. Litterst and A. M. Guarino

Other Investigators: R. L. Dedrick, R. L. Reagan, A. Leroy, J. R. Fouts and J. R. Bend

Cooperating Units: Biomedical Engineering and Instrumentation Branch, Division of Research Services, NIEHS

Man Years:

Total:	4.6
Professional:	2.3
Other:	2.3

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism, and excretion tend to dictate the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been initiated in the Drug Interaction Unit to investigate and appraise these parameters in a variety of animal species regarding drugs and other xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of action of many drugs. Only recently have data begun to accumulate which emphasize the important role of drug metabolism or biotransformation in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed *proximate toxicants*.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. *Acute Toxicity of Certain Foreign Chemical Compounds in Control and Phenobarbital Pretreated Mice*. The biological half-life of many drugs and other xenobiotics is determined to a large extent by the rates at which they are metabolized and/or excreted. Accordingly, the current pharmacological literature is replete with reports relating changes in drug metabolism measured *in vitro* to alterations in drug disposition or acute pharmacologic effects of these drugs. For example, it has been amply documented that the induction of hepatic microsomal mixed function oxidase (MFO) activity by pretreatment with phenobarbital is associated with a marked increase in the whole body clearance rates, decrease in the biological half lives and the duration of the acute pharmacologic effects of barbiturates such as hexobarbital and pentobarbital. Others have shown that phenobarbital pretreatment enhances the biliary excretion of certain agents. These increases in drug metabolism and excretion that accompany enzyme induction are of importance not only in chronic toxicity studies in animals but also in human chemotherapy. On the other hand, only a limited number of studies have appeared which have attempted to correlate changes in the rates of *in vitro* metabolism of drugs with possible changes in the acute toxicity of the same drugs.

The proposition that altered activity of microsomal enzymes measured *in vitro* may reflect altered *in vivo* acute toxicity has never been adequately tested for the substrates routinely used to monitor MFO activity *in vitro*.

Twenty-four hour LD_{50} values and slopes were determined in control and in phenobarbital pretreated male mice for several drugs routinely used to assay for *in vitro* activity of microsomal drug metabolizing enzymes. Phenobarbital pretreatment produced the expected increase in microsomal enzyme activity but had no effect on the LD_{50} of aniline, aminopyrine, p-nitrophenol or antipyrine. A 30% increase in the LD_{50} of ethylmorphine was produced by the phenobarbital treatment. Phenobarbital pretreatment had no effect on the slopes of the LD_{50} curves.

B. *Hepatic and Extrahepatic Drug Metabolism in Several Common Laboratory Animal Species*. The literature contains widely discrepant reports concerning the relative MFO activity in hepatic and extrahepatic tissues of different laboratory species. On the one hand, very low levels of enzyme activity have been reported in the lungs and kidneys of rats, guinea pigs, and mice, while on the other hand, our laboratory and others have reported enzyme activities in rabbit lung microsomes equal to or slightly higher than the activities in rabbit liver. The present investigation was initiated in order to shed further light on this apparent species-related difference as indicated in Table 1.

TABLE 1

Ratio of Hepatic to Extrahepatic Drug Metabolism in Several Animal Species

PARAMETER	RAT		RABBIT		MOUSE		HAMSTER		GUINEA PIG	
	Lung/Liver	Kidney/Liver	Lung/Liver	Kidney/Liver	Lung/Liver	Kidney/Liver	Lung/Liver	Kidney/Liver	Lung/Liver	Kidney/Liver
Cytochrome P-450	0.07	0.13	0.14	0.13	0.15	0.33	0.07	0.13	0.10	0.23
NADPH Cytochrome c Reductase	0.29	0.28	0.62	0.22	1.22	0.71	0.35	0.22	0.41	0.25
Hydroxylases										
Aniline	0.05	0.05	0.33	0.05	0.20	0.13	0.19	0.15	0.25	0.05
Biphenyl	0.13	0.05	1.06	0.12	0.25	0.05	0.35	0.18	0.20	0.05
Aminopyrine Demethylase	0.30	0.06	0.15	0.05	0.05	0.06	0.20	0.05	0.07	0.05
Glutathione S-aryl Transferase	0.05	0.05	0.24	0.24	0.29	0.48	0.08	0.10	0.08	0.13
N-acetyl Transferase										
PABA	0.33	1.00	1.11	0.39	0.25	0.94	0.15	0.47	0.34	1.32
Sulfadiazine	1.00	0.05	0.01	0.05	0.05	0.05	0.05	0.05	0.05	0.05
UDP-Glucuronyl-transferase										
PNP	0.18	0.75	0.06	0.44	0.68	0.32	0.18	0.36	0.05	0.37
OAP	0.22	0.89	0.13	0.05	0.60	0.20	0.63	0.19	0.36	0.44

It is obvious from the data presented that no single animal species is universally desirable for studies of extrahepatic and hepatic drug metabolism. For example, the mouse is relatively deficient in hepatic cytochrome P-450 and NADPH cytochrome c reductase but is nearly unexcelled in its extrahepatic complement of these components. In addition, no single species can be said to be most active with respect to any single organ. The rabbit liver, for example, provides the highest activity in the UDP-glucuronyltransferase pathway toward two different substrates but is quite poor in relative ability to demethylate or hydroxylate substrates while the rabbit pulmonary system is unexcelled in all activities.

C. Effects of Activators of UDP-Glucuronyltransferase Activity in Lung and Liver. A wide variety of diverse materials have been demonstrated to activate hepatic UDP-glucuronyltransferase activity when added *in vitro* to enzyme incubations. In several instances, the combination of two or more activators produces additive or even synergistic effects. The precise molecular mechanisms responsible for such activation are not known but it has been established that in the presence of activators, latent enzyme activities can be unmasked and expressed, activities which in the "native" state would not be measurable.

As an extension of the project described in Section B above, we studied the effects of a number of activators on UDP-glucuronyltransferase activities in rabbit liver and lung. In particular, we were interested in whether or not the very low or undetectable activities in lung (see above) could be activated and whether the patterns of activation might be different in the two organs.

Microsomal fractions prepared from rabbit livers and lungs were employed as the enzyme sources. The following materials were used as potential activators, each in a wide range of concentrations so as to determine the optimum concentration for activation: mersalyl, digitonin, cetyl trimethyl ammonium bromide (CETAB), sodium deoxycholate (DOC), Triton X-100, sodium dodecyl sulfate (SDS), diethylnitrosamine (DEN), uridine diphosphate-N-acetylglucosamine (UDPNAG), and Mg^{++}. The effects of the activators were studied alone and in various combinations. Because previous reports had indicated differential effects based on the substrate employed for measuring UDP-glucuronyltransferase activity two substrates were employed, viz, *p*-nitrophenol and *o*-aminophenol. The salient findings of this project may be summarized as follows:

(1) All activators exhibited biphasic effects as a function of concentration, i.e. activation at low concentrations and inhibition at higher concentrations.

(2) Mersalyl, digitonin and Triton X-100 were the most effective activators in liver. The effects of the detergents and UDPNAG were essentially additive. DEN reduced this activation. UDPNAG alone activated less than its combination with a detergent.

(3) There were no significant differences in the effects of activators alone or in combination, with the two different substrates, *p*-nitrophenol or *o*-aminophenol

(4) None of the activators, alone or in combination unmasked measurable UDP-glucuronyltransferase activity in lung. This was true with both substrates.

D. Comparison of Drug Toxicity and Drug Metabolism in Several Primate and Subprimate Species. It has been recognized for at least five years that looming on the horizon was an impending under-supply of rhesus monkeys for use in biomedical research. Given the heavy reliance that has been placed on the rhesus monkey in drug-development programs and particularly in the development of oncolytic agents, it was deemed desirable to attempt to identify an alternative species to the rhesus for possible use in the Cancer Program. In conjunction with Battelle Columbus Laboratories (BCL) through the Toxicology Program Office (TPO), personnel from the Drug Interactions Unit of the Laboratory of Toxicology devised an experimental design to attempt to identify such a species. Five animal species were selected for study and in order to accomodate possible sex differences, both males and females were scheduled for study. The species were: rhesus monkey, squirrel monkey, tupaia (a primitive primate species), mini-pigs and rats. The design involved three major subdivisions, each of which could be pursued independently, yet the data could be finally assembled in a way that all three components could be compared. The subdivisions were: (1) protocol toxicologic evaluation of four selected antineoplastic agents (Adriamycin, DTIC, Ara C, Cytoxan), (2) in vivo metabolism of the same four oncolytic drugs (to provide data on qualitative differences in drug metabolism), and (3) in vitro metabolism of a number of model drug substrates by hepatic preparations, both microsomal and post-microsomal supernatant fractions (to provide data on quantitative differences in drug metabolism). The experiments on points 1 and 2 above are to be conducted exclusively by BCL employees whereas the experiments in part 3 are being conducted jointly by BCL and LT personnel at the BCL facility. As of this writing, LT personnel have made three trips to the BCL facility for such purposes and this segment of the work is half-completed. It is anticipated that all of the experimental work in section 3 will be concluded by approximately August-September, 1975.

E. Drug Metabolism By Guinea Pig Hepatoma. The Line 10 hepatoma is a transmissible tumor which can be carried intraperitoneally in an ascites form or transplanted intradermally. The latter form will grow locally and in 3-4 weeks, will metastasize to regional lymph nodes and to the liver, lung and other organs. We examined a number of parameters of drug metabolism in this tumor. Very low levels of enzyme activity were detected in cell fractions prepared from the tumor (< 10% those of host liver). The ascites form appeared to have slightly higher levels of activity than the solid (intradermal) form. Both forms of the tumor were refractory to the inducing effects of phenobarbital whereas host liver responded to this treatment as did control (non-tumor-bearing) liver.

F. Disposition of Cis-diamino-dichloroplatinum (CDDP) in Dogs. In conjunction with Drs. Andre LeRoy and Robert Dedrick of Biomedical Engineering and Instrumentation Branch, Division of Research Services we have investigated the disposition of CDDP following its intravenous injection (1 mg/kg) in beagle dogs. Assay was for total Pt on wet digested tissue extracts using an atomic absorption technique. Plasma levels decayed biphasically with estimated half-lives of approximately 1 and 42 hours respectively. The only parenchymatous organs which retained significant quantities of drug were kidney (particularly cortex) and liver. Binding of the drug to plasma proteins was demonstrated using standard ultrafiltration techniques and the nature of the binding appeared to change as a function of time.

G. Antineoplastic Agents as Possible *In Vitro* MFO Substrates. For the past 1 to 2 years, we have examined certain antineoplastic agents for their *in vitro* metabolism of microsomal enzymes with the aim of finding an agent suitable for routine use for MFO activity. We began our search with compounds that were reported in the literature to be metabolized *in vitro* and others which we felt had a good potential to be demethylated to yield formaldehyde as one product. We have thus far screened at least the following drugs. Thalicarpine (NSC-68,075), coralyne sulfoacetate (NSC-154,890), hexamethylmelamine (NSC-13,875), adriamycin (NSC-123,127), puromycin (NSC-3,055), cytembena (NSC-104,801), DTIC (NSC-45,388), nitidine chloride (NSC-146,397) and 6-methyl mercaptopurine riboside (NSC-40,744). Our results have indicated that the compounds are metabolized, but to a very slight degree (i.e. 50 to 100 times less than our currently utilized substrates). While rechecking literature values we discovered relatively large absolute values (i.e. 30 to 80) but when units were considered (μg/150 mg fresh weight/90 minutes) the specific activity of the metabolic reaction was comparable to our own. Although we will continue to pursue this project, it would appear that the presently available anticancer drugs are poor substrates for *in vitro* microsomal metabolism.

H. Xenobiotic Distribution and Metabolism in Marine Species. For the past several years we have been exploring the possibility of using the dogfish shark as a model species for the rapid accrual of pharmacologic information concerning antineoplastic agents and other xenobiotics. The major rationale for this approach has been that both the rate of metabolism and rate of disposition of compounds administered to these animals might be lower than that of mammals. Within the past two years about 30 drugs and other xenobiotics have been tested in this system. Last year emphasis was placed on three model compounds of differing disposition patterns:

(1) Indocyanine green - a non-metabolized drug where the hepatic compartment is the sole one.

(2) BSP - a drug metabolized by glutathione conjugation and the hepatic compartment is dominant while the renal one is of minor importance.

(3) Phenol red - a drug metabolized by glucuronide conjugation and the renal and hepatic compartments are equally important in its overall disposition.

In addition, rather complete distribution studies were done for the antineoplastic agents Platinum (NSC-119,875) and Gallium (NSC-15,200).

1. *In Vivo* Distribution and Metabolism. The simultaneous biliary and urinary excretion of a model phenolic compound, phenol red and its major metabolite, a glucuronide, were measured in intact and in biliary fistulized dogfish sharks, *Squalus acanthias*. It was found that phenol red is extensively metabolized to its glucuronide conjugate by the dogfish shark and that both forms of the drug are actively transported into bile and urine. These conclusions were based on the following: both compounds are transferred from plasma to urine and bile against a large concentration gradient; the transfer process is saturable most easily in the renal compartment

but also in the hepatic compartment; and both excretion routes for the free drug and the glucuronide are inhibited by probenecid. In comparing intact vs. biliary fistulized animals, there were no significant differences in the 48 hour biliary excretion of total (free + glucuronide) phenol red, but the urine of intact fish contained two-fold more total drug than did fistulized animals. For a variety of reasons it was concluded that this species is of excellent potential value in studying the distribution and metabolism of anticancer agents. Some of these reasons follow: (1) the dogfish shark is slower to excrete and metabolize xenobiotics - this is very important for antineoplastic agents because mammals often excrete and metabolize these drugs too rapidly to measure accurately. (2) The hepatic and urinary transport systems of these fish and mammals are qualitatively similar, hence the relative importance of these excretion routes can be rapidly assessed. (3) This species is abundant and inexpensive.

2. In Vitro Metabolism. MFO activity toward aniline HCl, d-benzphetamine HCl, 7-ethoxycoumarin and benzpyrene by microsomes from various tissues of crustacea, cyclostomes, elasmobranchs and teleosts was studied. No activity was detectable in rock crab or lobster hepatopancreas microsomes, possibly because of inhibition by digestive juice. Activity per mg of hepatic microsomal protein in the other species was about one-tenth that seen in rat liver microsomes, although cytochrome P-450 levels were about 25% of those seen in rat or rabbit liver microsomes. Dogfish shark hepatic microsomal 7-ethoxycoumarin de-ethylase began to be inactivated at temperatures over 30-32°C while mouse and rabbit enzymes showed no evidence of inactivation at temperatures up to 45°C. Winter flounder and fundulus hepatic microsomal benzpyrene hydroxylase activities were ten times higher than those assayed in the other species; winter flounder hepatic microsomal benzpyrene hydroxylase was not as inducible as that in skate liver. The kidney was the only extrahepatic tissue found to contain significant microsomal mixed-function oxidase activity in the species studied. For most of the marine species studied, the rate at which the drug metabolizing pathways operate confirmed the in vitro data on phenol red conjugations mentioned above, i.e. fish use the same pathways of metabolism as do mammals, but do so with considerably less efficiency.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a subtreshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we plan to study the metabolic fate of antineoplastic agents. Pharmacokinetic studies of the dogfish shark model have only begun and will continue in greater detail, particularly for those anticancer agents just entering clinical trials.

Keyword Descriptors:

Xenobiotics
Drug metabolism
Drug distribution
Drug clearance
Microsomes
Conjugation
Oncolytic agents
Comparative pharmacology
Marine pharmacology
Biochemical toxicology

Honors and Awards:

Charter Member to The Cousteau Society

Publications:

1. Gram, T.E., Litterst, C.L. and Mimnaugh, E.G.: Comparative Aspects of Drug Metabolism by Mammalian Lung and Liver: Mixed Function Oxidation and Conjugation Reactions. In Morselli, P.L., Garattini, S. and Cohen, S.N. (Eds.) Drug Interactions. New York, Raven Press, 1974, pp. 103-110.
2. Mimnaugh, E.G., Litterst, C.L. and Gram, T.E.: The Effects of *In Vitro* Activators on Rabbit Lung and Liver Microsomal UDP-glucuronyltransferase Activity. Biochem.Pharmacol. (in press), 1975.
3. Pohl, R.J., Bend, J.R., Guarino, A.M. and Fouts, J.R.: Hepatic Microsomal Mixed-function Oxidase Activity of Several Marine Species from Coastal Maine. Drug Metabol. Disp. 2: 545-555, 1974.
4. Oliverio, V.T. and Guarino, A.M.: Absorption, Protein Binding, Disposition and Excretion of Antineoplastic Drugs. Biochem.Pharmacol. 2: 9-20, 1974.
5. Litterst, C.L., Mimnaugh, E.G., Cowles, A.C., Gram, T.E. and Guarino, A.M.: Distribution of ^{14}C-CCNU Derived Radioactivity Following IV Administration to the Rabbit in Three Potential Clinical Formulations. J.Pharm.Sci. 63: 1718-1721, 1974.
6. Litterst, C.L., Miller, E.: Distribution of Lindane in Brains of Control and Phenobarbital Pretreated Dogs at the Onset of Lindane-Induced Convulsions. Bull.Env.Cont.Toxicol. (in press), 1975.
7. Litterst, C.L.: Mechanically Self-induced Muzzle Alopecia in Mice. Lab.Anim.Sci. 24: 806-809, 1974.
8. Crawford, R.B. and Guarino, A.M.: Effects of DDT in Fundulus: Studies on Toxicity, Fate and Reproduction. Arch.Environm.Contam.&Toxicol. (in press), 1976.

Serial No. Z01 CM 06123-03 LT
1. Laboratory of Toxicology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computer Methods for Quantification of Structure-Activity Relationships

Previous Serial Number: NCI-6123

Principal Investigators: A. M. Guarino

Other Investigators: H. Fales, L. Hodes, G. W. A. Milne, M. B. Shapiro and J. M. Venditti

Cooperating Units: Drug Research and Development, Division of Cancer Treatment, Division of Computer Research and Technology, Laboratory of Chemistry and National Heart and Lung Institute

Man Years:

Total:	0.5
Professional:	0.5
Others:	None

Project Description:

Ever since Meyer and Overton observed a correlation between lipid solubility and anesthetic potency of a series of drugs, many other physical or structural parameters have been studied in an effort to discern other possible relationships between physical and structural features and a given pharmacologic effect. During the past year, one major project in this area has been concluded while most of the other projects were terminated.

Principal Findings:

The possible relationship between mass spectra and pharmacologic activity was investigated with the help of various computer-based pattern-recognition techniques. By means of these techniques a datum point can be located on grid coordinates, and when many of these points are related in some way, they will cluster. In our experiments, designed to develop the necessary models, we studied the relationships between the mass spectra and the accepted pharmacologic activities of several drug classes. The present study is a small part of a binary classification tree we have constructed for this and future projects. The first classification starts with the question of whether or not a drug acts on the nervous system. If it does, then we ask if it is a stimulant; if not, then it is a depressant. This latter group is further classified as sedatives or tranquilizers and other groups are similarly categorized as narcotics, analgesics, hallucinogens, antineoplastic agents, etc. If one attempts to find whether some arbitrary unknown drug sample belongs to drug class A or B, a binary tree can be made from two binary classifiers in series. Classifier 1 is to distinguish "A and B" from "Not A and Not B". Only the unknowns that cluster with "A and B" are then sent to classifier 2. In order to build up an additional classifier 1, a training set consisting of 53 drugs of various pharmacological activities as a class (Not A, B) and 64 other drug samples were run through the learning machine. When a series of unknowns was studied, 85% of them were correctly classified. The mapping techniques, both non-linear mapping and projection, should enable humans, who are far better pattern recognizers than computers, to be involved in decision-making as to which drugs are worthy of expensive and time-consuming biologic testing. It is believed that this kind of man-machine interaction is likely to yield more productive results than a system in which either the computer or biologic testing alone is required to make the final decision. It is noteworthy that pattern recognition techniques do not involve any prior knowledge of chemical structures or mass spectral rules. Therefore, although mass spectra were used as examples above, it is clear that the methods are general in scope and may be used as a tool for research on relationships which exist among any kinds of data which are not obviously correspondent. The fact that computer methods may predict pharmacologic activity of drugs from their mass spectra alone suggests that the foregoing is a new way of initial drug screening.

Significance to Biomedical Research and the Program of the Institute:

Although the Drug Development Program makes extensive use of computers in the acquisition, storage and retrieval of information on the antineoplastic drugs, studies have just been started to use computers as learning and predictive devices. The present studies are based on the hypothesis that since the ultimate mechanism by which a drug exerts its pharmacologic or toxicologic action is by interaction of the drug molecules with macromolecular receptors in the body, then the mass spectral fragments pattern or some other measure of molecular fragments, may indicate more precisely which parts of the drug molecule are necessary for the biologic action. Therefore, it is thought that once certain characteristics which are associated with good anticancer activity are established, the following can be done: 1) use such a system to establish priorities for biologic testing; 2) predict drug activity from the structure; 3) estimate which compounds from a series of analogs would have the greatest oncolytic activity; 4) suggest to the chemist which structural features should be built into a drug molecule to obtain maximum antineoplastic activity; and 5) substitute "toxicity" data for "pharmacologic activity" and thus develop knowledge for understanding which structural moieties are toxic. According to (5), then one should be able to predict and synthesize drugs designed to minimize toxicity and enhance therapy.

Proposed Course:

The mass spectra portion of this study was terminated about a year ago because of lack of personnel and equipment support. Correlations will be sought between molecular fragments and biological activity with the aim of selecting those molecular features most prominently associated with oncolytic effects. Such studies may discover that other drugs may have properties suggesting they should be tested for possible greater clinical activity than those already in the clinic.

Keyword Descriptors:

Mass spectra
Machine learning
Artifical intelligence
Cybernetics
Systems analysis
Drug screening
Pattern recognition
Non-linear mapping
Biological activity

Publication:

1. Guarino, A.M.: The Systems Approach, Cybernetics and Toxicology. In Pharmacological Basis of Cancer Chemotherapy. Baltimore, MD, Williams & Wilkins Co., 1975, pp. 343-350.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

July 1, 1974 - June 30, 1975

Studies in the Laboratory of Chemical Pharmacology are concerned with elucidation of the pharmacological properties of both potential and established antitumor agents. This includes the disposition, mechanism of action, structure-activity relationships, toxicity reversal, rational design and dose-response relationships of antitumor drugs and other model foreign compounds in laboratory animals and man. In many instances this requires the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. Of special interest in disposition studies is the establishment of rates and rate constants which govern the passage of drugs across membranes from one body compartment to another, the assessment of drug biotransformation or binding to cellular constituents or macromolecules, and the determination of the rate of elimination of the drug and its metabolites. When this information is considered together with a drug's chemical and physical properties and its route and schedule of administration, predictions can be made concerning the effective concentration of a drug or its metabolites in tumor and susceptible normal tissue. These studies are particularly important since one of the major factors contributing to an inadequate drug response in the host is the failure of the drug to reach the target site in sufficient concentration to eradicate the tumor cells. The knowledge gained from such studies when considered together with biochemical, toxicologic, and cytokinetic data will enable the clinician to utilize drug dosages and schedules for the most effective treatment of human malignancies.

In addition, another major effort of the laboratory is to develop methods for the determination of specific products of tumor cells; routine analysis of such products in body fluids would be valuable for diagnostic purposes and for the assessment of the antitumor effects of various therapeutic modalities.

During the past year a number of research projects were continued and several new studies were initiated. Some of the experimental findings are summarized below.

Studies of the folic acid antagonists continue in this laboratory; during the past year the diglutamate metabolite of methotrexate, MTX(G_1) (4-amino-4-deoxy-N^{10}-methylpteroylglutamyl-γ-glutamate), was synthesized and found to have inhibitory activity equal to the parent compound for dihydrofolate reductase from murine L1210 leukemia cells, and for the replication of L1210 cells _in vitro_ and _in vivo_. The enzyme-inhibitory and cytotoxic activity of the metabolite was about 11-fold greater than anticipated from earlier studies utilizing microbiological assay systems. The results indicate that MTX(G_1) formed _in vivo_ may contribute significantly to the pharmacological activity of the parent drug.

A differential sensitivity of various tissues (small intestine, bone marrow, tumor) to MTX has been demonstrated by maintaining constant plasma concentrations of the drug in mice. *In vitro* studies showed that this differential sensitivity was not due to differences among the tissues in affinity of dihydrofolate reductase for MTX. These results suggest a membrane transport-related selective response or a selective sensitivity in individual tissue biochemical pathways. The finding that deoxyuridine incorporation into DNA is most rapidly inhibited in small intestine, then femur marrow, and lastly tumor, under conditions of constant MTX infusion, suggest selective permeability differences. Studies with constant infusions of MTX into mice also permitted evaluation of the recovery of certain biochemical systems after complete inhibition. These investigations indicated a difference in the rate at which different tissues recovered after complete inhibition of either deoxyuridine or thymidine incorporation into DNA. These rates of recovery could not be related to permeability differences, as suggested by the earlier rates of inhibition, or to growth rate. These findings suggest that there are differential rates of recovery of select biochemical pathways in different tissues and that these differences might be exploited for a more beneficial chemotherapeutic response with timed exposure to such agents as citrovorum factor and thymidine.

Three studies have been carried out in patients receiving MTX: 1. studies of the plasma pharmacokinetics of MTX administered intrathecally, 2. pharmacokinetic studies in patients with ovarian cancer and 3. pharmacokinetic studies in patients receiving high-dose MTX. In studies of the plasma pharmacokinetics of methotrexate administered intrathecally, it was found that plasma concentrations remained at pharmacologically significant levels ($> 10^{-8}$M) more than twice as long after an intrathecal dose as after the same oral or intravenous dose. These observations may provide a rational basis for the generally held clinical impression that the bone marrow and gastrointestinal toxicity of intrathecally administered methotrexate exceeds that of methotrexate administered by oral or intravenous routes.

MTX pharmacokinetics and the time-course of inhibition of ^{3}H-UdR incorporation into DNA have been examined in 6 patients with ovarian carcinoma. Each received a standard dose of 25 mg/m^2 i.v., following which serial samples of plasma and ascites fluid were taken for MTX determination, and samples of bone marrow for *in vitro* measurement of ^{3}H-UdR incorporation. The MTX plasma disappearance curve had 3 components, the second and third phases showing half-lives of 23 hours and 12.1 hours, respectively. Preferential accumulation of drug in ascites was observed in all patients, probably secondary to slow efflux from the large pool of ascitic fluid. Large ascitic effusions were associated with delayed MTX disappearance from plasma. The bone marrow of patients with ovarian cancer recovers from MTX-induced inhibition of DNA synthesis at plasma levels of 3×10^{-8}M, while DNA synthesis in ascites cells was only briefly inhibited by MTX in 5 of 6 patients despite high levels in the ascites. The apparent biochemical resistance of these cells to MTX is currently under closer study.

Because of the serious, and at times fatal, toxicity associated with high dose methotrexate, its pharmacokinetics were studied in patients receiving doses of 50 mg/kg or greater by infusion over a 6 hr period. Peak plasma levels of 0.1 mM or greater were found, but these levels fell rapidly at the end of the infusion; the plasma disappearance curve showed at least two components, with half lives of 2 and about 12 hours respectively. Urinary methotrexate was 10-fold higher than plasma methotrexate despite diuresis, and the drug was shown to reach levels above the limits of its solubility in acid urine, indicating the need for hydration and alkalinization. A probable sequence leading to toxicity was defined, including renal precipitation of drug, failure of tubular secretion, prolonged plasma retention, and severe myelosuppression. This sequence is being studied in non-human primates.

Disposition (absorption, distribution, metabolism and excretion) studies were undertaken with several new potential antitumor agents. Among the agents investigated were methanesulfon-m-anisidide, 4'-(9-acridinyl-9-amino) monohydrochloride (MeAc), gallium, coralyne sulfoacetate, and inosine dialdehyde. In addition the role of the lymphatic system in the disposition of anticancer agents was investigated.

Plasma radioactivity following ^{14}C-MeAc administration was found to be associated with parent compound, a metabolite, and a compound that was bound to plasma proteins and was not removed by TCA precipitation or Sephadex gel filtration. The kinetics of each of these components was followed in rats after several different i.v. and oral doses. The plateau phase of the plasma disappearance curve described in the previous report is a result of protein-bound MeAc. The metabolite was actively transported into bile and the transport system saturated at therapeutic doses given orally or i.v. At higher doses of MeAc (10 mg/kg, i.v.), biliary flow was inhibited and plasma radioactivity increased due to an accumulation of metabolite. Tissue distribution studies in mice indicated highest levels of radioactivity to be associated with liver. MeAc interacts with sulfhydryl-containing compounds *in vitro* under physiological conditions, yielding acridone.

The pharmacokinetics of gallium is under investigation in man, using Ga^{67} as a tracer, in conjunction with the phase I study of this agent. Plasma disappearance curves are biphasic with an initial rapid component followed by a much more gradual decline. Excretion is primarily via the urine, with 26 to 32% eliminated during the first 24 hours; 2.2 to 5.4% was eliminated in the feces during the first 48 hours.

Studies on the pharmacological disposition of the alkaloid, coralyne sulfoacetate (NSC-154890), were initiated in mice and rats. In both species the major route of excretion was biliary following either i.p. or i.v. administration. In rats, 25-40% of the ^{14}C was eliminated in the bile in the first 24 hours, and only 2% in the urine. Transport into the bile appeared to be active and the transport system saturable. A minor portion of the biliary ^{14}C was associated with a metabolite. No sex differences were found with regard to plasma concentrations of the drug, percent excreted in the bile, or percent eliminated as metabolite.

After treatment with various radioactive antitumor agents, the appearance of the parent compounds as well as their labeled metabolites in lymph of thoracic-duct cannulated rats has been measured. Results thus far have demonstrated that for most of the compounds examined, the lymphatic channels represent only a minor route of absorption regardless of whether the compounds were administered intra-duodenally, subcutaneously or intramuscularly. Thus, following treatment with radioactive CCNU, methyl-CCNU, guanazole, diethylstilbestrol, testosterone, estradiol or dibromomannitol, 5% or less of the administered dose was recovered in thoracic duct lymph during the 24 hours following treatment. This figure probably reflects the distribution of these compounds in total body water, of which lymph is a part; it is comparable to that obtained with antipyrine, which is known to distribute in body water. The only antitumor agent examined thus far which appears to enjoy preferential lymphatic absorption is o,p-DDD; 20-35% of the administered dose of radioactivity was recovered in thoracic duct lymph, and 70% of this radioactivity was identified as the parent compound. Studies on the lymphatic absorption and distribution of other antitumor agents, including ara-C palmitate, phenestrin, dexamethasone, cytoxan and azathioprine, are in progress.

Tissue distribution studies following i.v. administration of ^{14}C-inosine dialdehyde to mice indicated no preferential uptake of radioactivity by any tissue; however, there was a transient increase in the kidney, possibly because urinary excretion is its major route of elimination. A chemical assay was developed that makes use of the chemical reactivity of the two aldehyde groups and is specific for free inosine dialdehyde in quantities as low as 1 μg in urine or plasma. A comparison of the results of this assay with those obtained by the radioactive tracer technique indicated that all of the radioactivity eliminated in the first 6 hours in dogs (50% of dose) can be recovered as unchanged inosine dialdehyde. This is consistent with chromatographic data and with the finding that inosine dialdehyde is not a substrate for aldehyde oxidase.

Investigations were undertaken to elucidate the antitumor activity and mechanism of action of several drugs including maytansine, tilorone, interferon, inosine dialdehyde and methanesulfon-*m*-anisidide, 4'(9-acridinyl-9-amino) monohydrochloride (MeAc).

Maytansine is a compound with antitumor activity, recently isolated by Kupchan and his co-workers from the East African plants *Maytenus serrata* and *Maytenus buchananii*. It belongs to the ansa macrolide class of compounds, characterized by an aromatic nucleus to which a macrocyclic aliphatic bridge is attached at two non-adjacent positions. The growth of leukemia L1210, L5178Y and P388 cells in suspension culture was found to be inhibited by concentrations of maytansine in the nanomolar range. Histological examination of L1210 cells after a 24-hr exposure to $10^{-8}M$ maytansine showed 30% with mitotic figures, as compared to 3% for control cells. In studies on the effects of maytansine on macromolecular synthesis, DNA synthesis was found to be inhibited to a greater extent than RNA or protein synthesis. In *in vivo* studies, maytansine showed antitumor activity at a dose-level of 0.025 mg/kg/day against the P388 murine leukemia, but failed to show activity against a vincristine-resistant line of the same tumor.

Tilorone hydrochloride, a synthetic low molecular-weight inducer of interferon, showed significant in vivo antitumor activity against the Walker 256 carcinosarcoma in rats, two experimental reticulum cell sarcomas, and the murine leukemias K1964 and YLL. It was without activity against a number of other murine leukemias, including leukemia L1210. In in vitro studies, tilorone was an effective inhibitor of three out of four DNA polymerases isolated from Walker 256 carcinosarcoma cells, and of RNA-directed DNA polymerase from Rauscher leukemia virus ("reverse transcriptase").

Mouse interferon was found to have antitumor effects against the transplantable tumor A-RCS but not against leukemia L1210 or P388. The mechanism of its antitumor activity is not a direct cytotoxic effect, since interferon per se is not active in vitro, against leukemia L1210, L5178Y or P388. Further studies in progress will help to define the mechanism of this agent.

Inosine dialdehyde, an antitumor agent undergoing Phase I clinical trial, has two aldehyde functional groups; both groups were found to interact with primary amino groups of proteins, forming complexes that do not dissociate under physiological conditions. When albumin (M.W. 70,000) was incubated with inosine dialdehyde and chromatographed on Sephadex G-200, protein elutes in the void volume, suggesting a M.W. exceeding 800,000. This indicates intermolecular cross-linking by inosine dialdehyde at multiple sites on the proteins, resulting in aggregate formation. Amino acids can be attached to proteins in the same manner, can inhibit the attachment of drug to protein, and can reverse the toxicity of inosine dialdehyde in tissue culture. The periodate-oxidation products of several other nucleosides were also found to possess protein cross-linking properties.

Interaction of MeAc with double stranded calf-thymus DNA results in a shift in the absorption spectrum of MeAc. The magnitude of the spectral shift was used to determine the extent of interaction. MeAc inhibition of E. coli DNA-dependent RNA polymerase is due both to a direct interaction with the enzyme and by interaction with DNA, with subsequent alteration of template function. The metabolite of MeAc isolated from rat bile did not interact with DNA (either single-stranded or double-stranded). MeAc interacts with protein forming a complex that does not dissociate by Sephadex gel filtration.

Previously initiated studies on the two enzymes carboxypeptidase G_1 and cytidine deaminase were continued. Carboxypeptidase G_1 has proved valuable as a means of identifying α-peptidase glutamate groups in folate analogs such as metabolites of methotrexate esters and polyglutamates. In addition, preliminary testing of carboxypeptidase G_1 in man have indicated the ability of this enzyme to deplete circulating folates, to cleave circulating methotrexate, and have demonstrated only one instance of hypersensitivity. The main effort currently is to obtain sufficient enzyme to investigate its utility as a rescue agent following high dose methotrexate in man.

Cytidine deaminase and deoxycytidine kinase have been purified and characterized from human leukemic cells; and their levels have been determined in an untreated population of 30 patients with AML and 10 patients with CML, blastic crisis. Wide variations in enzyme activity were observed in the leukemic cells, and a positive correlation has been seen thus far between high levels of kinase, low levels of deaminase, and a favorable response to ara-C therapy.

Studies on the embryotoxic and cytogenetic effects of hycanthone have been completed. Hycanthone is embryolethal and teratogenic in both mice and rabbits. In the former species, teratogenic effects are most marked on day 7 of gestation, whereas embryolethal activity is found on day 6, 7 and 8. Hycanthone depresses DNA and RNA synthesis in cultured human leucocytes, and on the basis of in vivo ^{3}H-thymidine uptake studies, inhibits DNA synthesis in 7-day mouse embryos. Hycanthone induces chromosomal aberrations in cultured human leucocytes; however, no chromosome abnormalities are apparent in cells of fetal or maternal liver or in maternal marrow following hycanthone exposure.

Studies of the embryotoxic and cytogenetic effects of adriamycin are currently in progress. Preliminary results indicate that when given to pregnant mice on day 7 of gestation, adriamycin is highly active over an extremely narrow range of doses. At 6 mg/kg, it causes a small increase in embryolethality and teratogenicity as compared to controls, at 8 mg/kg it causes death of almost all of the fetuses, and at 10 mg/kg 100% maternal mortality occurs. Studies are underway to define the teratogenic dose of adriamycin and to determine whether a "sensitive period" exists for its teratogenic and embryolethal effects.

The embryotoxic and cytogenetic effects of maytansine are being examined in pregnant mice, and preliminary results indicate that this compound is a potent teratogen. When given to pregnant mice on day 7 of gestation, maytansine exerts dose-related embryolethal and teratogenic effects over a range of 4 doses from 100-250 μg/kg; at 1,000 μg/kg maternal deaths occur.

The availability of a large breeding and experimental monkey colony (600 animals representing 5 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, and biochemical studies in subhuman primates. Seven of the chemicals employed have induced tumors to date in the three species of Old World monkeys under study - these include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP) and dipropylnitrosamine (DPNA], methylnitrosourea, cycasin and its aglycone (MAM-acetate), aflatoxin B_1 and procarbazine. Methylnitrosourea has induced squamous cell carcinomas of the mouth, esophagus and pharynx; procarbazine has induced acute myelogenous leukemias, hemangiosarcomas and lymphoma. The other carcinogens have all induced primary liver cell carcinoma in Old World monkeys.

DENA has induced primary liver tumors in 81% and 100% of Old World monkeys treated by oral and i.p. routes, respectively; 82% of monkeys given PIP orally developed tumors, as did all monkeys receiving i.p. injections of

DPNA. The other compounds tested also induced liver tumors, although they possessed less potent carcinogenic activity than the nitrosamines. Thus 4/22 monkeys (18%) receiving MAM acetate i.p. and 3/35 monkeys (9%) receiving aflatoxin B_1 orally or i.p. developed liver carcinoma. Alpha-fetoprotein (AFP) levels in sera of monkeys with biopsy-proven tumors were measured using radioimmunoassay. The wide range of values (a 100,000-fold variation) is analogous to that seen in patients with hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine-induced neoplasms had significantly higher serum levels of AFP than those with tumors induced by 1-nitrosopiperidine, MAM acetate or aflatoxin B_1. Serial determinations of AFP levels in monkeys with liver tumors demonstrated a tendency for the values to plateau after reaching a level characteristic for that tumor.

Monkeys with primary liver tumors are being utilized in a search for materials of possible value in the x-ray diagnosis of primary and secondary liver tumors in humans. Results to date indicate that ^{67}gallium and several experimental X-ray contrast materials are useful, both in indicating the location and size of liver tumors in monkeys and in evaluating the effects of antineoplastic agents on the growth of the tumors.

Methods have been developed to evaluate whether or not specific markers are elevated in the blood or urine of cancer patients. Initially, patients with advanced disease were entered in these studies. To date specific tRNA nucleosides and polyamines have been found to be elevated in the urine of more than 50% of patients with several different types of solid tumors. Three markers, carcinoembryonic antigen, human chorionic gonadotropin and N^2,N^2-dimethylguanosine have been found elevated in 97% of advanced breast cancer patients. tRNA Nucleosides, polyamines, and the β-aminoisobutyric acid/β-alanine ratio appear to be particularly useful for following patients with Burkitt's lymphoma. Preliminary studies indicate that carcinoembryonic antigen may be useful in following patients with bronchogenic carcinoma, breast cancer and gastrointestinal malignancy. Further developmental work is underway and clinical studies projected. If successful, biologic markers may be of assistance in evaluating patients with cancer during treatment, and in determining recurrence of disease following surgery, radiation or chemotherapy.

In addition to the basic laboratory research effort, each senior investigator shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with development of analytical-biochemical methodology; the study of pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and *in vitro* predictive systems; the development of pharmacokinetic mathematical models; and the development of biological markers for detection of cancer and assessment of cancer treatment. Approximately 12 new antitumor agents are studied each year, each of which reflect the interest of the Division of Cancer Treatment Program area for developing antitumor drugs for the clinic.

Project No. Z01 CM 03502-12 LCP
1. Lab. of Chemical Pharmacology
2. Pharmacokinetics and Pharmacodynamics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Folic Acid Antagonists in Experimental Solid Tumors *In Vivo*

Previous Serial Number: Same

Principal Investigator: Daniel S. Zaharko

Other Investigators: Robert L. Dedrick and Robert J. Lutz

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years:

Total:	1.8
Professional:	1.3
Others:	0.5

Project Description:

The Lewis Lung solid tumor system in mice is notably resistant to treatment with methotrexate. This model system is therefore being used to investigate high dose methotrexate treatment alone and in combination with other drugs in attempts to understand the reasons for the tumors resistance. In an extension of this work by contract with George Washington University, Contract No. NO1-CM-12206, the spontaneous canine lymphosarcoma is being studied simultaneously using similar techniques.

Studies in mice in which the plasma concentration of methotrexate (MTX) has been maintained constant at various levels indicate that different tissues (small intestine, bone marrow, tumor) respond differently to the same MTX concentration. *In vitro* studies of these same tissues, dihydrofolate reductase binding affinity to MTX indicate no differences. These results suggest a membrane transport related selective response or an unknown sensitivity in individual tissue biochemical pathways. The finding that deoxyuridine incorporation into DNA is most rapidly inhibited in small intestine, then femur marrow and lastly tumor under conditions of constant MTX infusion suggest selective permeability differences. These findings agree with earlier studies (1973-1974) in which the pharmacokinetics of MTX transport indicated that small intestine permeability was greater than that of femur marrow in the rat.

Studies with constant infusions of MTX into mice have also permitted us to follow the recovery of certain biochemical systems after complete inhibition. These investigations indicate that there is a difference in the rate at which different tissues recover after complete inhibition of either deoxyuridine or thymidine incorporation into DNA. These rates of recovery can neither be related to the expected permeability differences as suggested by the earlier rates of inhibition nor to the expected recovery as a function of growth rates (cell kinetics). These findings suggest that there are differential rates of recovery of select biochemical pathways in different tissues and that these differences might be exploited for a more beneficial chemotherapeutic response with timed exposure to such agents as citrovorum factor and thymidine.

Since we have had such success, with the constant infusion of MTX in mice, in gaining further insight into its mechanism of action in the in vivo situation, we plan to continue these studies in combination with other drugs and with select antidotes such as citrovorum factor and thymidine to gain further knowledge in attempts to improve treatment of solid tumors.

Keyword Descriptors:

Methotrexate; citrovorum factor; thymidine rescue; solid tumors; Lewis Lung carcinoma; pharmacokinetics; mice-constant drug infusion

Publications:

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Project No. Z01 CM 03505-12 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Potential Embryotoxic, Carcinogenic and Other Adverse Effects of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Susan Sieber Fabro

Other Investigators: Jacqueline Whang-Peng

Cooperating Units: Cytogenetic Oncology Section, Medical Oncology, NCI

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

Information on the susceptibility of newborn and adult animals to environmental chemical carcinogens and particularly to drugs of interest in cancer chemotherapy, including the antiviral agents, is of vital necessity and of particular relevance to the program of the NCI. The characterization and treatment of such induced tumors is of both theoretical and practical importance. The specific objectives are:

To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy, such as alkylating agents, antimetabolites, agents that intercalate with DNA, polymers and various antiviral agents and antibiotics.

To investigate the potential embryotoxic effects of anticancer agents and antiviral agents that may be used in patients of child bearing age with or without tumors.

To ascertain unusual pharmacologic and toxic properties of various antibiotics and antiviral agents which are used in patients with neoplastic disease.

Major Findings:

Embryotoxicity of Antitumor Agents

Studies on the embryotoxic and cytogenetic effects of hycanthone have been completed. Hycanthone is embryolethal and teratogenic in both mice and rabbits. In the former species, teratogenic effects are most marked on day 7 of gestation, whereas embryolethal activity is found on days 6, 7 and 8. Hycanthone depresses DNA and RNA synthesis in cultured human leucocytes, and on the basis of *in vivo* ^{3}H-thymidine uptake studies, inhibits DNA synthesis in 7-day mouse embryos. Hycanthone induces chromosomal aberrations in cultured human leucocytes; however, no chromosome abnormalities are apparent in cells of fetal or maternal liver or in maternal marrow following hycanthone exposure.

Studies of the embryotoxic and cytogenetic effects of adriamycin are currently in progress. Preliminary results indicate that when given to pregnant mice on day 7 of gestation, adriamycin is highly active over an extremely narrow range of doses. At 6 mg/kg, it causes a small increase in embryolethality and teratogenicity as compared to controls, at 8 mg/kg it causes death of almost all of the fetuses, and at 10 mg/kg 100% maternal mortality occurs. Studies are underway to define the teratogenic dose of adriamycin and to determine whether a "sensitive period" exists for its teratogenic and embryolethal effects. In addition, samples of maternal and fetal liver and marrow from adriamycin-treated mice are being examined for possible cytogenetic damage, and fetal and maternal hearts prepared for electron microscopic examination.

Evaluation of Agents for Carcinogenic Activity in Mice

Procarbazine is under further evaluation as a carcinogen in several species both alone and in combination with other antitumor agents and X-radiation. This is similar to the combination chemotherapy protocol for patients with Hodgkin's disease. Procarbazine has been found to induce tumors in mice, rats and monkeys in our colony and may be suspected of being carcinogenic in humans when administered chronically.

Hycanthone is presently under study as a potential carcinogen in CDF$_1$ mice. Hycanthone is under evaluation because it is widely used in South America and Africa for the treatment of schistosomiasis and over 100,000 people have been exposed to the compound, and because it produces embryotoxic effects in the mouse and rabbit and chromosomal aberrations in human PHA-stimulated lymphocytes at high doses. The mice autopsied thus far, 1 year after dosing ceased, did not have tumors and thus there is little carcinogenic potential of hycanthone in mice.

Several interferon inducers including poly I·poly C and tilorone have been further evaluated for potential carcinogenic effects. Data thus far suggest that these compounds are not highly carcinogenic in CDF$_1$ mice.

Adverse Effects of Anticancer Agents in Humans

Examination and summarization of the literature, patient data at the NCI and studies of patient specimens have led us to conclude the following with regards to the deleterious effects of cancer chemotherapeutic agents in humans.

All three classes of antitumor compounds (alkylating agents, antimetabolites and antitumor antibiotics) that have been studied extensively *in vitro* and *in vivo* induce chromosomal aberrations; these abnormalities vary according to the agent and the test system used. Combinations of agents or agents plus irradiation also produce cytogenetic damage in human cells. However, the long-term consequences of such damage cannot be ascertained at the present time.

The alkylating agents caused oligospermia or azoospermia when given to lymphoma patients. This effect was usually related to the total dose administered and was reversible only in some cases. Combination chemotherapy also exerted adverse effects on male fertility, but all such combinations included an alkylating agent. There is little information on the possible adverse effects of the antimetabolites, antitumor antibiotics and miscellaneous synthetic agents on spermatogenesis. In addition to their effects on the male reproductive system, the alkylating agents (primarily chlorambucil and cytoxan) produce amenorrhea in the female. The initial action appears to be arrest of follicular maturation followed by decreased estrogen secretion and finally primary ovarian failure.

The human conceptus appears to be more resistant to the teratogenic effects of anticancer agents than one would predict from animal studies. Thus, although most of the commonly-used antitumor agents exert embryotoxic and/or teratogenic effects in animals, only the folic acid antagonists can be implicated with certainty as teratogens in the human. However, there are indications that some of the alkylating agents (chlorambucil, cytoxan and busulfan) may cause embryotoxicity or malformations in humans.

The appearance of more than 93 tumors in recipients of renal homografts during immunosuppression with steroids and imuran suggests that azathioprine has carcinogenic activity; however, this is a complex problem requiring more documentation and experimentation, especially in non-human primates. The increasing use of cancer chemotherapeutic and immunosuppressive agents in the treatment of non-neoplastic disorders has provided some data that also implicates chlorambucil and cytoxan as having carcinogenic potential in humans. Although no anticancer agents can as yet be implicated with certainty as being carcinogenic in the human, the agents more frequently associated with neoplasia are chlorambucil, melphalan, thio-TEPA and combination chemotherapy plus radiotherapy. Further studies in animals, especially non-human primates, and careful monitoring of all patients treated with antineoplastic agents will provide a more accurate picture of the carcinogenic potential of these drugs.

Keyword Descriptors:

Embryotoxic; teratogenic; chromosomal aberrations; azoospermia; amenorrhea; carcinogenic; hycanthone; adriamycin

Publications:

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Project No. Z01 CM 03506-12 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pharmacology and Disposition of Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Richard Cysyk, Susan Sieber Fabro and Jacqueline Plowman

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, immunologic and histopathologic techniques.

Major Findings:

Disposition of NSC-141549 (MeAc), Methanesulfon-m-Anisidide, 4'-(9-Acridinyl-9-Amino) Monohydrochloride

Plasma radioactivity following ^{14}C-MeAc administration was associated with parent compound, a metabolite, and a portion that was bound to plasma proteins and is not removed by TCA precipitation or Sephadex gel filtration. The kinetics of each of these components was followed in rats after several different IV and oral doses. The plateau phase of the plasma disappearance curve described in the previous report is a result of protein-bound MeAc. The metabolite was actively transported into the bile and the transport system saturated at therapeutic doses given orally or IV. At higher doses of MeAc (10 mg/kg, IV) biliary flow was inhibited and plasma radioactivity increased due to an accumulation of metabolite. Tissue distribution studies in mice indicated highest levels of radioactivity to be associated with liver. MeAc interacts with sulfhydryl containing compounds *in vitro* under physiological conditions, yielding acridone.

Disposition of NSC-118994 (Inosine Dialdehyde)

Tissue distribution studies following IV administration of ^{14}C-inosine dialdehyde to mice indicated no preferential uptake of radioactivity by any tissue; however, there was a transient increase in the kidney due, possibly, to urinary excretion being the major route of elimination. A chemical assay was developed that makes use of the chemical reactivity of the two aldehyde groups and is specific for free inosine dialdehyde at quantities as low as 1 µg in urine or plasma. A comparison of the results of this assay with those obtained by the radioactive tracer technique indicated that all of the radioactivity eliminated in the first 6 hours (50% of dose) in dogs can be recovered as unchanged inosine dialdehyde. This is consistent with chromatographic data (see previous report) and with the finding that inosine dialdehyde is not a substrate for aldehyde oxidase as might be expected.

Gallium Disposition Studies

The pharmacokinetics of gallium is under investigation in man using Ga^{67} as a tracer. This is in conjunction with the phase I study of this agent. Plasma disappearance curves are biphasic with an initial rapid component followed by a much more gradual decline. Excretion is primarily via the urinary route with 26 to 32% eliminated during the first 24 hours while 2.2 to 5.4% was eliminated in the feces during the first 48 hours.

Disposition of Coralyne Sulfoacetate

Studies on the pharmacological disposition of the alkaloid, coralyne sulfoacetate (NSC-154890), were initiated in mice and rats. In both species the major route of excretion was biliary following either i.p. or i.v. administration. In rats, 25-40% of the ^{14}C was eliminated in the bile in the first 24 hours, and only 2% in the urine. Transport into the bile appeared to be active and the transport system saturable. A minor portion of the biliary ^{14}C was associated with a metabolite. No sex differences were found with regard to plasma concentrations of the drug, percent excreted in the bile or percent eliminated as metabolite.

Studies on the Role of the Lymphatic System in Disposition of Antitumor Agents

After treatment with various radioactive antitumor agents, the appearance of the parent compounds as well as their labeled metabolites in the lymph of thoracic-duct cannulated rats has been measured. Results thus far have demonstrated that for most of the compounds examined, the lymphatic channels represent only a minor route of absorption regardless of whether the compounds were administered intra-duodenally, subcutaneously or intramuscularly. Thus, following treatment with radioactive CCNU, methyl-CCNU, guanazole, diethylstilbestrol, testosterone, estradiol or dibromomannitol, 5% or less of the administered dose was recovered in thoracic duct lymph during the 24 hours following treatment. This figure probably reflects the distribution of these compounds in total body water, of which lymph is a part; it is comparable to that obtained with antipyrine, which is known to distribute in body water. The only antitumor agent examined thus far which appears to enjoy preferential lymphatic absorption is o,p-DDD; 20-35% of the administered dose of radioactivity was recovered in thoracic duct lymph, and 70% of this radioactivity was identified as the parent compound. Studies on the lymphatic absorption and distribution of other antitumor agents, including ara-C palmitate, phenestrin, dexamethasone, cytoxan and azathioprine, are in progress.

Ara-C-5'-Methyl Phosphonate

Ara-C-5'-methyl phosphonate was synthesized as a possible antimetabolite to circumvent Ara-C resistance that results from deletion of deoxycytidine kinase. It was found to be far less toxic than Ara-C against L-1210 *in vitro*. Its interaction with certain key enzymes is under investigation.

Keyword Descriptors:

Inosine dialdehyde; gallium; coralyne sulfoacetate; methanesulfon-*m*-anisidide, 4'-(9-acridinyl-9-amino) monohydrochloride; lymphatic absorption; drug disposition.

Publications:

1. Cysyk, R. L.: Proposed new derivatives of cytosine arabinoside (NSC-63878) to circumvent resistance. *Cancer Chemother. Rep.* 58: 298-299, 1974.

2. Cysyk, R. L.: Chemical assay for the anti-tumor agent inosine dialdehyde (NSC-118994) in biological fluids. Cancer Chemother. Rep., in press.

3. Sieber, S. M., Cohn, V. H., and Wynn, W. T.: The entry of foreign compounds into the thoracic duct lymph of the rat. Xenobiotica 4: 265-284, 1974.

4. Adamson, R. H.: Daunomycin (NSC-82151) and adriamycin (NSC-123127): a hypothesis concerning antitumor activity and cardiotoxicity. Cancer Chemother. Rep. 58: 293-294, 1974.

5. Adamson, R. H., and Oliverio, V. T.: Farmacologia Chemioterapica Antitumorali. In Enciclopedia della Chimica, Vol. III. Florence, Italy, USES Edizoni Scientifiche, 1974, pp. 297-300.

6. Adamson, R. H., Canellos, G. P., and Sieber, S. M.: Studies on the antitumor activity of gallium nitrate and other group IIIa metal salts. Cancer Chemother. Rep., in press.

Project No. Z01 CM 03509-12 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

Previous Serial Number: Same

Principal Investigator: Richard H. Adamson

Other Investigators: Susan Sieber Fabro, T. Phillip Waalkes, Robert McIntire, Pelayo Correa and Dan Dalgard

Cooperating Units: Laboratory of Cell Biology, NCI; Department of Pathology, Louisiana State University, New Orleans, Louisiana; and Hazleton Laboratories, Inc., Falls Church, Virginia

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic in man and to develop model primate tumor systems predictable for therapy for man and for development of biological markers. Specifically the objectives are:

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To study the effects of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To develop model non-human primate tumor systems.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

To evaluate the long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

Methods Employed:

Usual pharmacologic, biochemical, clinical, anatomical pathologic, cytological, and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years.

Major Findings:

General Information:

The present colony consisting of 600 animals is comprised of five species: Macaca mulatta, Macaca fascicularis, Cercopithecus aethiops, Galago crassicaudatus, and Aotus trivirgatus. Of these 450 are experimental; the remaining 150 are adult breeders which supply the newborns for experimental study.

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed, the hematological, biochemical and diagnostic parameters monitored and information concerning the methods utilized in treating the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

No major disease problems have been encountered during this period.

Compounds Inducing Tumors:

The summary below is for chemicals that have induced tumors in Old World monkeys. Seven of the chemicals employed have induced tumors to date in the three species of Old World monkeys under study - these include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP), and dipropylnitrosamine (DPNA)], methylnitrosourea (MNU), cycasin and its aglycone MAM acetate, aflatoxin B_1, and procarbazine. Methylnitrosourea has induced squamous cell carcinomas of the mouth, esophagus and pharynx and procarbazine has induced acute myelogenous leukemias, hemangiosarcomas and lymphoma while the other carcinogens have all induced primary liver cell carcinomas in Old World monkeys.

DENA given by the oral route has induced primary liver tumors in 30/37 monkeys while when given by the i.p. route induced liver tumors in 80/80 Old World monkeys. When PIP was given orally 9/11 monkeys developed tumors for an incidence of 82%. Six of six monkeys treated i.p. with DPNA developed primary liver tumors and the incidence of monkeys developing liver cancer while receiving compounds other than the nitrosamines was less. Thus 4/22 monkeys (18%) receiving MAM acetate i.p. and 3/35 monkeys receiving aflatoxin B_1 orally or i.p. (9%) developed liver carcinoma.

The carcinogen studied most extensively is DENA. When given intraperitoneally in gradually increasing doses of 10, 20 and 40 mg/kg every 14 days a tumor incidence of 100% can be anticipated. The average induction time is 15 months with a range of 6 to 22 months.

The AFP response in these animals as determined by double diffusion is quite variable in that some animals become positive after 5-6 months of compound administration, whereas others may take 10-15 months. Once they are positive however, the AFP level escalates rapidly. Exploratory laparotomies done on 30 animals shortly after the first positive AFP determination revealed no gross evidence of tumor. Histologically, toxic hepatitis and hyperplastic nodules were commonly seen at this stage, but tumors were not detected.

The gross and histologic picture of early tumor varies from 1-2 isolated nodules surrounded by relatively normal liver to numerous small nodules, cirrhosis, toxic hepatitis and necrosis. Tumor generally continues to invade the remaining liver until the entire organ is replaced by tumor. Metastasis to the lungs is common in animals with advanced tumor, whereas metastatic tumor in other locations occurs infrequently. Death or euthanasia because of unmanageable tumor generally occurs by 24 months of age. The AFP commonly reaches levels of 5000 μg/ml at this point. If the level of DENA is maintained at 20 mg/kg or lower, tumor induction-time is longer, tumor growth rate is reduced, and AFP levels are of a lower magnitude. Three groups of six animals each are being treated, at 1, 5, and 10 mg/kg in an attempt to determine if there is a carcinogenic threshold dose for DENA.

The serum levels of AFP using radioimmunoassay in biopsy-proven disease were scattered over a wide range of 100,000 fold variation, which is analogous to the range seen in human hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine induced neoplasms had significantly higher serum levels of AFP than those with tumors induced by 1-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations of AFP levels demonstrated a tendency for each monkey with liver tumor to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: 1. Serum from hepatoma monkeys injected i.v. into adult monkeys, 2. the post-partum decline in neonatal monkeys and 3. the decline in a hepatoma-bearing monkey following surgical removal of the tumor. Despite many obvious and subtle differences among these situations the results tended to corroborate a T 1/2 for AFP of 2.8-3.6 days. In both the neonatal and post-surgical removal of tumor there may be continued synthesis of AFP, perhaps declining in the neonatal monkeys and increasing in the monkey which may develop recurrent tumor post-surgery.

Further work on catabolic rates of AFP where there is a varied total body load and establishment of rates of cellular synthesis will allow the estimation of total body tumor cell number and the changes that take place as a result of various modalities of tumor therapy.

Diagnostic Studies

Diagnostic studies for primary hepatocellular carcinoma which may be applicable to human primary and secondary liver cancer have continued using ^{67}gallium administered i.v. and experimental x-ray contrast materials administered i.v. and via the hepatic artery. Studies to date indicate that these materials may be useful in the diagnosis of primary and secondary liver tumors and for careful evaluation of effects of anticancer drugs on liver tumors.

Keyword Descriptors:

Non-human primates; carcinogenesis; procarbazine; nitrosamine; methylnitrosourea; cycasin; aflatoxin B_1

Publications:

1. McIntire, K. R., Adamson, R. H., Waldman, T. A., and Dalgard, D. W.: Metabolism of alpha-fetoprotein in non-human primates. In Masseyeff, R. (Ed.): Alpha-Feto-Protein. Paris, France, Institute National de la Sante et de la Recherche Medicale, 1974, pp. 301-312.

2. Dalgard, D. W., McIntosh, C. L., McIntire, K. R., Correa, P., and Adamson, R. H.: Hepatic carcinogenesis and serum alpha-fetoprotein in non-human primates. In Masseyeff, R. (Ed.): Alpha-Feto-Protein. Paris, France, Institute National de la Sante et de la Recherche Medicale, 1974, pp. 211-216.

3. Ablashi, D. V., Armstrong, G. R., Easton, J. M., Chopra, H. C., and Adamson, R. H.: Evaluation of effects of cytosine arabinoside on Herpesvirus Saimiri replication in Owl monkey kidney cells. Biomedicine 21: 57-60, 1974.

4. Correa, P., Dalgard, D. W., and Adamson, R. H.: Olfactory neuroepithelioma in a Cynomolgus monkey (_Macaca fascicularis_). _J. Med. Primatology_ 4: 51-61, 1975.

Project No. Z01 CM 06108-06 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Susan Sieber Fabro and Richard L. Cysyk

Other Investigators: F. John Krolikowski and Sidney T. Yancey

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	1.5
Others:	1.5

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design antitumor agents that will inhibit metastases or tumor growth by affecting blood vessels and fibrin cross-linking.

To design new anticancer agents utilizing knowledge obtained so that a higher cell kill can be obtained and the problem of resistance can be circumvented.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied as well as effects on DNA, RNA and various proteins.

Major Findings:

Mechanism of Action of NSC-141549 (MeAc)-Methansulfon-m-Anisidide, 4'-(9-Acridinyl-9-Amino) Monohydrochloride

Interaction of MeAc with double stranded calf-thymus DNA results in a shift in the absorption spectrum of MeAc. The magnitude of the spectral shift was used to determine the extent of interaction. MeAc inhibition of *E. coli* DNA-dependent RNA polymerase is due both to a direct interaction with the enzyme and by interaction with DNA and subsequent alteration of template function. The metabolite of MeAc isolated from rat bile did not interact with DNA (either single-stranded or double-stranded). MeAc interacts with protein forming a complex that does not dissociate by Sephadex gel filtration.

Mechanism of Action of NSC-118994 (Inosine Dialdehyde)

Inosine dialdehyde, the periodate oxidation product of inosine, has two aldehyde functional groups both of which were found to interact with primary amino groups of proteins forming complexes that do not dissociate under physiological conditions. When albumin (M.W. 70,000) was incubated with inosine dialdehyde and chromatographed on Sephadex G-200, protein elutes in the void volume indicating a M.W. exceeding 800,000. This indicates intermolecular cross-linking by inosine dialdehyde at multiple sites on the proteins resulting in aggregate formation. Amino acids can be attached to proteins in the same manner, can inhibit the attachment of drug to protein and can reverse the toxicity of inosine dialdehyde in tissue culture. The periodate-oxidation products of several other nucleosides were found to also possess protein cross-linking properties.

Interferon

Mouse interferon was found to have antitumor effects against the transplantable tumor A-RCS but not against leukemia L1210 or P388. The mechanism of this antitumor effect is not a direct cytotoxic effect since interferon *per se* is not active in tissue culture. Further studies in progress will help to define the mechanism of this agent.

Tilorone

Tilorone hydrochloride, a synthetic low molecular-weight inducer of interferon, showed significant antitumor activity against the Walker 256 carcinosarcoma in rats, the reticulum cell sarcomas RCS J96132 and A-RCS in mice, and the murine leukemias K1964 and YLL. It was without activity *in vivo* against a number of other murine leukemias, including leukemia L1210. In *in vitro* studies, tilorone was an effective inhibitor of three out of four DNA polymerases isolated from Walker 256 carcinosarcoma cells, and of RNA-directed

DNA polymerase from Rauscher leukemia virus ("reverse transcriptase"). In preliminary studies, tilorone (50 mg/kg) was found to be without significant teratogenic properties when given to pregnant mice on days 5-7, 8-10, or 11-13 of gestation, although a significant degree of embryotoxicity was observed.

Transglutaminase

Studies with transglutaminase will continue and in particular the determination of transglutaminase activity in various tissues will be completed. The possibility of metastasis being greater in tissues with high contents of transglutaminase is not only of clinical importance, but will also increase our knowledge of transglutaminase and tumor growth.

Keyword Descriptors:

Interferon; tilorone; transglutaminase; inosine dialdehyde, methanesulfon-*m*-anisidide, 4'-(9-acridinyl-9-amino) monohydrochloride; mechanism of action

Publications:

1. Adamson, R. H., Ablashi, D. V., Cicmanec, J. L., and Dalgard, D. W.: Chemotherapy of *herpesvirus saimiri* induced lymphoma-leukemia in the Owl monkey. *J. Med. Primatol.* 3: 68-72, 1974.
2. Yancey, S. T., and Bleyer, W. A.: An acute lymphocytic leukemia arising in a CDF_1 mouse: the characteristics and response to certain cancer chemotherapeutic agents. *Cancer Res.* 34: 1866-1869, 1974.
3. Johns, D. G., Sieber, S. M., and Adamson, R. H.: Studies on the antitumor activity of tilorone. *J. Natl. Cancer Inst. Monogr.*, in press.

Project No. Z01 CM 06120-04 LCP
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetic Profile on Drug Action In Vivo

Previous Serial Number: Same

Principal Investigators: Bruce A. Chabner, Robert C. Young and Charles E. Myers

Other Investigators: Daniel S. Zaharko, Joan Bull, Robert Pinedo, Ronald Stoller, and Samuel Jacobs

Cooperating Units: Medicine Branch, NCI

Man Years:

Total: 3.5
Professional: 2.5
Others: 1.0

Project Description:

Objectives:

In using any pharmacologic agent, certain critical information is needed in order to design an optimal schedule of drug administration. One must know:

1) The nature and kinetics of distribution and disposition of the agent.
2) Its mechanism of action.
3) The levels of parent drug or active metabolite required for the desired effect on target tissue.

While extensive information is now accumulating with regard to pharmacokinetics and mechanism of action of the common antitumor drugs, little is known about the drug concentrations which produce toxicity and antitumor effects in vivo. This important consideration is the object of our research efforts. We have chosen methotrexate as a model agent for study because it is easily measured and active as the parent compound. Secondly, its mechanism of action, the inhibition of dihydrofolate reductase, with consequent block of the conversion of dUMP to dTMP allows one to follow its action in vivo by determining the incorporation of ^{3}H-UdR into DNA.

We have also extended our studies to 5-fluorouracil and have made similar correlations between intracellular levels of F-dUMP and dUMP and inhibition of thymidylate synthesis by 5-FU. These studies will be described in the annual report for the Medicine Branch, NCI, in further detail.

Methotrexate Studies

Methods Employed:

Methotrexate (MTX) levels were measured as previously described by the standard dihydrofolate reductase inhibition assay which allows quantitation of 10^{-9}M or greater MTX, and by a newly devised competitive protein binding assay employing the same enzyme as a binding protein.

Major Findings:

Determination of MTX pharmacokinetics and time course of inhibition of ^{3}H-UdR incorporation in patients with ovarian carcinoma. Six patients have been studied, each receiving a standard dose of 25 mg/m^2 IV. Thereafter serial *in vitro* studies of ^{3}H-UdR incorporation into DNA were performed on bone marrow, and ascites samples and the results of these incorporation studies were correlated with the plasma and ascites levels of MTX. The detailed results of this study will be described by Dr. Robert Young in the Medicine Branch Annual Report (Z01 CM 03420). The major research findings regarding pharmacokinetics were a three phase disappearance from plasma with a 1/2 life of 2-3 hours for the 2nd phase and 12.1 hours for the third phase. Preferential accumulation of drug in ascites was observed in all patients, probably secondary to slow efflux from the large pool of ascitic fluid. Large ascitic effusions were associated with delayed MTX disappearance from plasma. The bone marrow of patients with ovarian cancer recovers from inhibition by MTX at plasma levels 3 x 10^{-8}M, while DNA synthesis in ascites cells was only briefly inhibited by MTX in 5 of 6 patients despite high levels in the ascites, indicating biochemical resistance of these cells to MTX. This latter subject is currently under closer study.

Pharmacokinetics of High Dose Methotrexate In Man

Because of the serious, and at times fatal toxicity associated with high dose methotrexate we have undertaken intensive study of methotrexate pharmacokinetics in patients receiving 50 mg/kg or greater infusions over a 6 hr period. Our primary findings include peak plasma levels of 0.1 mM or greater, rapid fall in levels after the infusion with t 1/2's of 120 minutes, and later, approximately 12 hrs. Urinary methotrexate was 10 fold higher than plasma despite diuresis, and the drug was shown to reach levels above the limits of its solubility in acid urine, indicating the need for hydration and alkalinization. A probable sequence leading to toxicity was defined, including 1) renal precipitation of drug, 2) failure of tubular secretion, 3) prolonged plasma retention, and 4) severe myelosuppression.

This sequence is being studied in primates.

A possible new metabolite of methotrexate has been identified in studies of patient urine, and will be the subject of further investigation.

Keyword Descriptors:

Pharmacokinetics; methotrexate; 5-fluorouracil; thymidylate synthetase; bone marrow, intestinal mucosa, scheduling

Publications:

1. Stoller, R. G., Jacobs, S., Drake, J., and Chabner, B. A.: The pharmacokinetics of high dose methotrexate. Cancer Chemother. Rep., July 1975, in press.

2. Chabner, B. A., and Slavik, M.: Prospectives on the clinical use of high dose methotrexate. Cancer Chemother. Rep., July 1975, in press.

3. Chabner, B. A., Coleman, C. N., Myers, C., and Johns, D. G.: The clinical pharmacology of antineoplastic agents. New Engl. J. Med., June 1975, in press.

4. Myers, C. E., Young, R. C., Johns, D. G., and Chabner, B. A.: Assay of 5-fluorodeoxyuridine 5'-monophosphate and deoxyuridine 5'-monophosphate pools following 5-fluorouracil. Cancer Res. 34: 2682-2688, 1974.

5. Myers. C. E., Young, R. C., and Chabner, B. A.: The role of dUMP pool elevation in recovery from inhibition of DNA synthesis by 5-fluorouracil. J. Clin. Invest., in press.

Project No. Z01 CM 06121-04 LCP
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Purification and Properties of Carboxypeptidase G_1

Previous Serial Number: Same

Principal Investigator: Bruce A. Chabner

Other Investigators: Joseph R. Bertino, David R. Makulu, David G. Johns, and Robert C. Young

Cooperating Units: Department of Pharmacology, Yale University School of Medicine; New England Enzyme Center of Tufts University; Medicine Branch, NCI

Man Years:

Total:	0.5
Professional:	0.2
Others:	0.3

Project Description:

Objectives:

1. To investigate prevention of methotrexate toxicity by Carboxypeptidase G_1.

Results:

Preliminary testing of Carboxypeptidase G_1 in man was undertaken in 1973-1974, and indicated the ability of this enzyme to deplete circulating folates, to cleave circulating methotrexate, and demonstrated only one instance of hypersensitivity in man. The main effort currently is to obtain sufficient enzyme to investigate its utility as a rescue agent following high dose methotrexate in man.

In addition, the enzyme has proved valuable as a means of identifying α-peptidase glutamate groups in folate analogs such as metabolites of methotrexate esters and poly glutamates.

Keyword Descriptors:

Carboxypeptidase; antifolate; methotrexate; enzyme therapy

Publications:

See Section on Studies on the Clinical Pharmacology of the Folate Acid Antagonists (Z01 CM 06129-01 LCP).

Project No. Z01 CM 06124-03 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biologic Markers Program for Clinical Cancer

Previous Serial Number: Same

Principal Investigator: T. Phillip Waalkes

Other Investigators: Douglas Tormey, Richard H. Adamson, Duane Lakings, Lawrence Broder and F. John Krolikowski

Cooperating Units: NCI-Breast Cancer Service; Solid Tumor Service; Pediatric Oncology Service; VA Lung Cancer Service; Mayo Clinic; Hoffmann La Roche, Oak Ridge National Laboratory and University of Missouri

Man Years:

Total:	2
Professional:	2
Others:	0

Project Description:

Objectives:

The objectives of this report are to develop and evaluate biologic markers which can be utilized as a means for following cancer patients during therapy, particularly chemotherapy. Multiple analytical methods are involved including high pressure liquid chromatography, gas liquid chromatography, GC-mass spectrometry, amino acid analyzer, and radioimmunoassay. Specific materials as potential markers under evaluation include carcinoembryonic antigen, human chorionic gonadotropin, tRNA nucleosides, polyamines, β-aminoisobutyric acid, β-alanine, Regan's isoenzyme, serum fucose, sialic acid, and urinary hydroxy proline/creatinine ratios. Studies involve the determination of levels in advanced (broad spectrum) malignant disease as compared to normal and disease controls, and also specific disease studies, e.g., markers in various stages of breast cancer, in metastatic lung cancer, in advanced gastrointestinal cancer, in Burkitt's lymphoma and in ovarian cancer. To date specific tRNA nucleosides and polyamines have been found elevated in the urine of greater than 50% of patients with several different solid tumor types. Three markers, carcinoembryonic antigen, human chorionic gonadotropin and N^2,N^2-dimethylguanosine have been found elevated in 97% of advanced breast cancer patients. tRNA nucleosides, polyamines, and β-aminoisobutyric acid/β-alanine ratio appear to be particularly useful for following patients with Burkitt's lymphoma. Carcinoembryonic antigen, in preliminary studies, may be useful in following patients with bronchogenic carcinoma, breast cancer as well as gastrointestinal malignancy. Further developmental work is underway and clinical studies projected. If successful biologic markers may be of great assistance in evaluating patients with cancer during treatment, and in determining recurrence of disease following surgery, radiation or chemotherapy.

Contracts As Direct Extensions:

1. Oak Ridge National Laboratory - development of special instruments and related analytical techniques which can be used to measure specific potential markers. Work has progressed on tRNA nucleosides and on serum glycoproteins. (Contract No. NCI-CR-71-60; Budget (1974) = $85,000).

2. University of Missouri - development of methods, especially using such instruments as gas liquid chromatography and amino acid analyzer, for sensitive determination of potential markers and also, of practical methods, which can be used for routine analyses. Currently carrying out specific studies on body fluid samples from patients in the various investigations underway. (Contract No. NIH-N01-CM-12323; Budget (1974) = $160,000).

3. Johns Hopkins University - an independent but related project evaluating specific biosynthetic methods for labeling specific globulins in mouse myeloma models for determination of *in vivo* synthetic rate. The latter will be correlated with total tumor cell number. (Contract No. N01-CM-43718; Budget (1974) = $37,589).

4. University of Arizona - an independent project, (1) appraisal of a method for determining total tumor cell number for patients with multiple myeloma during the course of their illness and as a means for evaluating therapeutic response; (2) development of a staging system, based on above method (a) using pre-treatment values, to predict eventual outcome of disease as related to total tumor cell burden, treatment, and specific clinical parameters. (Contract No. N01-CM-33713; Budget (1974) = $120,000).

Keyword Descriptors:

Biologic markers; high pressure liquid chromatography; mass spectrometry; amino acid analyzer; radioimmunoassay; carcinoembryonic antigen; human chorionic gonadotropin; tRNA nucleosides; polyamines; β-aminoisobutyric acid; β-alanine; Regan's isoenzyme; serum fucose; sialic acid; hydroxyproline; Burkitt's lymphoma.

Publications:

1. Gehrke, C. W., Kuo, K. C., Zumwalt, R. W., and Waalkes, T. P.: Polyamines in human urine by an automated ion-exchange method. J. Chromatogr. 89: 231-238, 1974.

2. Chang, S. Y., Lakings, D. B., Zumwalt, R. W., Gehrke, C. W., and Waalkes, T. P.: Quantitative determination of methylated nucleosides and pseudouridine in urine by gas-liquid chromatography. J. Lab. Clin. Med. 83: 816-830, 1974.

3. Mrochek, J. E., Dinsmore, S. R., and Waalkes, T. P.: Analytical techniques in the separation and identification of urinary degradation products of tRNA; application to the study of urines from cancer patients. J. Natl. Cancer Inst. 53: 1553-1563, 1974.

4. Tormey, D. C., Waalkes, T. P., Ahmann, D., Gehrke, C. W., Zumwalt, R. W., Snyder, J., and Hansen, H.: Biologic markers in breast cancer. I. Incidence of abnormalities of CEA, HCG, three polyamines, and three minor nucleosides. Cancer, in press.

5. Waalkes, T. P., Gehrke, C. W., Bleyer, W. A., Zumwalt, R. W., Olweny, C. L. M., Kuo, K. C., Lakings, D. B., and Jacobs, S.: Potential biologic markers in Burkitt's lymphoma. Cancer Chemother. Rep., in press.

6. Waalkes, T. P., Gehrke, C. W., Tormey, D. C., Zumwalt, R. W., Heiser, J., Lakings, D. B., Ahmann, D., and Moertel, C. G.: The urinary excretion of polyamines by patients with advanced cancer. Submitted to J. Natl. Cancer Inst.

7. Levine, L., Waalkes, T. P., and Stolbach, L.: Serum levels of N^2,N^2-dimethylguanosine and pseudouridine as determined by radioimmunoassay for patients with malignancy. J. Natl. Cancer Inst., in press.

Project No. Z01 CM 06125-03 LCP
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pharmacology of Maytansine, Dimethylbenzylrifampicin, and Other Ansa Macrolides

Previous Serial Number: Same

Principal Investigator: David G. Johns and Richard H. Adamson

Other Investigators: Mary K. Wolpert, Susan Sieber Fabro, Richard L. Cysyk and Jacqueline Whang-Peng

Cooperating Units: Molecular Biology and Methods Development Section, Drug Evaluation Branch, DR & D, NCI and Cytogenetic Oncology Section, Medical Oncology, DCT, NCI

Man Years:

Total:	1.6
Professional:	1.4
Others:	0.2

Project Description:

Maytansine is a compound with antitumor activity recently isolated by Kupchan and his co-workers from the East African plants *Maytenus serrata* and *Maytenus buchananii*. It belongs to the ansa macrolide class of compounds, characterized by an aromatic nucleus to which a macrocyclic aliphatic bridge is attached at two non-adjacent positions. The objective of this project is to elucidate the mode of action and whole animal pharmacology of this and other ansa macrolides, in order to provide information of potential value in clinical studies.

The growth of L1210, L5178Y and P388 cells in suspension culture was found to be suppressed by concentrations of maytansine in the nanomolar range. Histological examination of L1210 cells after a 24-hr exposure to 10^{-8}M maytansine showed 30% with mitotic figures, as compared to 3% for control cells. In studies on the effects of maytansine on macromolecular synthesis, DNA synthesis was found to be inhibited to a greater extent than RNA or protein synthesis. In *in vivo* studies, maytansine showed antitumor activity at a dose-level of 0.025 mg/kg/day against the P388 murine leukemia, but failed to show activity against a vincristine-resistant line of the same tumor.

The embryotoxic and cytogenetic effects of maytansine are being examined in pregnant mice, and preliminary results indicate that this compound is a potent teratogen. When given to pregnant mice on day 7 of gestation, maytansine exerts dose-related embryolethal and teratogenic effects over a range of 4 doses from 100-250 μg/kg; at 1,000 μg/kg maternal deaths occur. The effects of maytansine on mitotic indices, chromosome morphology, and macromolecular biosynthesis of cultured human leucocytes is being investigated, as well as its *in vivo* cytogenetic effect in pregnant mice and their fetuses.

These results are of particular interest to the program of the Institute since maytansine is the first agent of the ansa macrolide class to show significant antitumor activity. Studies of its ability to arrest tumor cells at a specific phase of mitosis are being actively pursued by a variety of cytological and biochemical methods. Similar studies will also be extended to other compounds of this class, as they become available.

Keyword Descriptors:

Maytansine; ansa macrolide; rifampicin; mitotic arrest; vincristine; DNA synthesis

Publications:

1. Wolpert-Defilippes, M. K., Adamson, R. H., Cysyk, R. L., and Johns, D. G.: Initial studies on the cytotoxic action of maytansine, a novel ansa macrolide. *Biochem. Pharmacol.* 24: 751-754, 1975.

2. Wolpert, M. K., Lu, K., Derr, C. J., Loo, T. L., and Johns, D. G.: Physiological disposition of 2,6-dimethyl-4-benzyl-4-demethylrifampicin in rats, mice, and dogs. *Drug Metab. Disposition* 2: 237-246, 1974.

Project No. Z01 CM 06126-03 LCP
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Purification and Properties of Cytidine Deaminase

Previous Serial Number: Same

Principal Investigator: Bruce A. Chabner

Other Investigators: Ronald G. Stoller, C. Norman Coleman, and Paul Chang

Cooperating Units: Hematology and Supportive Care Branch; Plasmaphoresis Laboratory; Medicine Branch; Baltimore Cancer Research Center

Man Years:

Total:	2
Professional:	1.5
Others:	0.5

Project Description:

Objectives:

1. To purify and characterize the enzymes cytidine deaminase and deoxycytidine kinase found in normal and malignant leukocytes.

2. To elucidate the mechanism of enzyme regulation and the effect of ara-C treatment.

3. To determine the relationship between enzyme levels and response to therapy.

Methods Employed and Major Findings:

Cytidine deaminase and deoxycytidine kinase have been purified and characterized from human leukemic cells; and their levels have been determined in an untreated population of 30 patients with AML and 10 patients with CML, blastic crisis. Wide variations in enzyme activity were observed in the leukemic cells, and a positive correlation has been seen thus far between high levels of kinase, low levels of deaminase, and a favorable response to ara-C therapy.

Further biochemical studies have:

1. Effectively separated deaminase and kinase on hydroxylappatite columns.
2. Demonstrated the separate nature of uridine-cytidine kinase and deoxycytidine kinase by hydroxylappatite chromatography.
3. Purified human liver cytidine deaminase 150-fold and shown its identity with the myeloblast deaminase.
4. Proved the competitive nature of inhibition of hepatic cytidine deaminase by tetrahydrouridine (K_i 1 x 10^{-7}M).
5. Begun work on the purification and properties of deoxycytidylate deaminase in human leukemic cells.
6. Shown the absence of a deoxycytidine phosphorylase in human granulocytes, thus discounting a possible mechanism of 5-fluorocytosine activation in man.

Keyword Descriptors:

Cytosine arabinoside; cytidine deaminase; deoxycytidine kinase; AML; 5-azacytidine; granulocytes

Publications:

1. Coleman, C. N., Johns, D. G., and Chabner, B. A.: Studies on mechanisms of resistance to cytosine arabinoside: Problems in the determination of related enzyme activities in leukemic cells. Annals N. Y. Acad. Sci., in press.

Project No. Z01 CM 06127-01 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Modified Degradation Products of Normal Monkey Liver and Hepatoma tRNA

Previous Serial Number: None

Principal Investigator: T. Phillip Waalkes

Other Investigators: Duane B. Lakings and Richard H. Adamson

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	1 1/4
Others:	1/4

Project Description:

Objectives:

tRNAs were isolated and purified from normal monkey liver and hepatomas induced by N-diethylnitrosoamine. Urine samples were also obtained prior to death of the monkeys. The tRNAs are being degraded and base or nucleoside analyses carried out depending on the mode of hydrolysis, by acid or enzymatically. By means of a special nucleoside and free base high pressure anion exchange liquid automated chromatography with UV-absorption for measurement, the free bases and/or the nucleosides are being analyzed in the tRNA and the modified (primarily methylated) nucleosides quantitated in the urine for correlation. β-aminoisobutyric acid and β-alanine as urine excretion products of pyrimidines as well as polyamines are also being analyzed in the urine. Comparisons between normal and hepatoma monkeys are being made. To date elevated amounts of modified nucleosides have been found in the hepatoma monkey urines.

Keyword Descriptors:

tRNA; hepatoma; N-Nitrosodiethylamine; high pressure liquid anion exchange chromatography; β-aminoisobutyric acid; β-alanine; polyamines; modified tRNA nucleosides or bases; methylated nucleosides or bases.

Publications: None

Project No. Z01 CM 06128-01 LCP
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pharmacologic Aspects of Pentamidine

Previous Serial Number: None

Principal Investigator: T. Phillip Waalkes

Other Investigators: David R. Makulu

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	1
Others:	1/2

Project Description:

Objectives:

Pentamidine is the drug of choice for treatment of *Pneumocystis carinii* infections, a pulmonary complication seen not infrequently in seriously ill cancer patients undergoing chemotherapy. In this situation, pentamidine can cause serious renal toxicity. Studies have shown that pentamidine accumulates in the kidney with gradual excretion as the intact drug. Further investigations over the past year have shown that pentamidine is an inhibitor of dihydrofolate reductase and in addition combines with nucleic acids and their degradation products to the level of nucleotides. These reactions may be factors both in the drugs activity but also its toxicity. No further work is intended on this project in the near future.

Keyword Descriptors:

Pentamidine; *Pneumocystis carinii*; dihydrofolate reductase; nucleic acids

Publications:

1. Waalkes, T. P., and Makulu, D. R.: Pharmacologic aspects of pentamidine. *Natl. Cancer Inst. Monogr.*, in press.
2. Makulu, D. R., and Waalkes, T. P.: Interaction between aromatic diamidines and nucleic acids. *J. Natl. Cancer Inst.* 54: 305-309, 1975.

Project No. Z01 CM 06129-01 LCP
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Clinical Pharmacology of Folic Acid Antagonists

Previous Serial Number: NCI-3502

Principal Investigator: David G. Johns

Other Investigators: Samuel A. Jacobs

Man Years:

Total:	2.2
Professional:	1.2
Others:	1.0

Project Description:

While methotrexate has been in clinical use as an antitumor agent for a number of years, the recent introduction of high-dose schedules followed by citrovorum factor rescue in the treatment of solid tumors has resulted in renewed interest in this agent, and has revealed also that our knowledge of the pharmacology of this and related folate antagonists is still incomplete. Among areas which still require study are the pharmacokinetics of high-dose methotrexate, the biological rôle of the recently-discovered diglutamate metabolite of the drug, and the pharmacology of intrathecally administered methotrexate.

The diglutamate metabolite of methotrexate, MTX(G_1) (4-amino-4-deoxy-N^{10}-methylpteroylglutamyl-γ-glutamate), was synthesized in this laboratory and found to be equal in activity to its parent compound, methotrexate, as an inhibitor of dihydrofolate reductase from murine L1210 leukemia cells, and as an inhibitor of the replication of L1210 cells in vitro and in vivo. The enzyme-inhibitory and cytotoxic activity of the metabolite was some 11-fold greater than anticipated from earlier studies utilizing microbiological assay systems. The results indicate that MTX(G_1) formed in vivo may contribute significantly to the pharmacological activity of the parent drug.

In studies of retained methotrexate activity present in liver samples obtained at necropsy from patients who had received the drug as an antineoplastic agent, significant MTX(G_1) could be detected; the MTX(G_1) to MTX ratio increased with the length of the interval after the last dose of methotrexate, with more than half the total dihydrofolate reductase-inhibitory activity being in the MTX(G_1) form at longer time-periods. No significant accumulation of higher polyglutamates of methotrexate could be detected.

In studies of the plasma pharmacokinetics of methotrexate administered intrathecally, it was found that plasma concentration remained at a pharmacologically significant level ($> 10^{-8}$M) more than twice as long after an intrathecal dose as after an identical oral or intravenous dose. These observations may provide a rational basis for the generally held clinical impression that the bone marrow and gastrointestinal toxicity of intrathecally administered methotrexate exceeds that of methotrexate administered by oral or intravenous routes.

In view of the still increasing interest in the clinical applications of methotrexate, studies on its pharmacology and toxicology will be continued. Particular emphasis will be placed on assessing the possible role of accumulation of the MTX(G_1) metabolite in the long-term toxicity of the drug in man.

Keyword Descriptors:

Methotrexate; methotrexate diglutamate; citrovorum factor; folate antagonists; pharmacokinetics; clinical pharmacology; dihydrofolate reductase

Publications:

1. Johns, D. G., Farquhar, D., Chabner, B. A., and McCormack, J. J.: Effect of carboxyl group esterification of methotrexate on its properties as an inhibitor of dihydrofolate reductase and a substrate for aldehyde oxidase. Biochem. Soc. Trans. 2: 602-604, 1974.

2. Jacobs, S. A., Bleyer, W. A., Chabner, B. A., and Johns, D. G.: Altered plasma pharmacokinetics of methotrexate administered intrathecally. Lancet I: 465-466, 1975.

3. Jacobs, S. A., Adamson, R. H., Chabner, B. A., Derr, C. J., and Johns, D. G.: Stoichiometric inhibition of mammalian dihydrofolate reductase by the γ-glutamyl metabolite of methotrexate, 4-amino-4-deoxy-N^{10}-methylpteroyl-γ-glutamate. Biochem. Biophys. Res. Commun. 63: 692-698, 1975.

4. Johns, D. G.: Metabolism of cancer chemotherapeutic agents via pathways utilized by endogenous substrates. In Sartorelli, A. C. and Johns, D. G. (Eds.): Antineoplastic and Immunosuppressive Agents I. (Handbook of Experimental Pharmacology 38/1). New York-Berlin-Heidelberg, Springer-Verlag, 1974, pp. 270-287.

5. Loo, T. L. and Johns, D. G.: Metabolism of antitumor agents. In Pharmacological Basis of Cancer Chemotherapy (M. D. Anderson Hospital 27th Annual Symposium on Fundamental Cancer Research). Baltimore, Williams and Wilkins, 1975, pp. 57-68.

6. Sartorelli, A. C. and Johns, D. G. (Eds.): Antineoplastic and Immunosuppressive Agents I (Handbook of Experimental Pharmacology 38/1). New York-Berlin-Heidelberg, Springer-Verlag, 1974, pp. 762.

ANNUAL REPORT

LABORATORY OF MOLECULAR PHARMACOLOGY

DIVISION OF CANCER TREATMENT

July 1, 1974 -- June 30, 1975

The function of the Laboratory of Molecular Pharmacology is to carry out studies that would aid our understanding of the reactions of drugs with targets within cells, the consequences of such reactions on the physiology of the cell, and how these effects could lead to a chemotherapeutic result.

The current program of the Laboratory can be considered in 3 categories: (1) studies of macromolecular damage and repair in various types of cells; (2) studies of the mechanisms of drug action on DNA replication; and (3) studies of potential cell culture models for chemotherapy.

The essence of a chemotherapeutic result is to produce a selective toxicity against an undesired cell type. Major possibilities for selective toxicity could arise from understanding of differences between cell types in the capacity for macromolecular damage and repair. The best established macromolecular target for chemotherapy is DNA. Available methods, however, have been inadequate to measure DNA damage at the level at which it is produced by pharmacologic treatments in mammalian cells. We have therefore put substantial effort into developing suitable methodology. We have developed a new technique which measures DNA single-strand breakage at levels of pharmacologic interest in mammalian cells and which is simple enough for large scale application. The basis of the procedure is to measure the rate of elution of DNA strands from lysed cells by alkali. The rate of DNA elution was found to be an extraordinarily sensitive indicator of single-strand breakage. During the past year, we have further studied the mechanism of DNA elution and have concluded that the rate-limiting step is the unwinding and disentanglement of DNA strands in the alkaline solution. Variations of the procedure were developed for studying newly replicated DNA segments and DNA crosslinking.

Single-strand DNA breaks were observed in mouse L1210 cells and human fibroblasts after treatment with various chemotherapeutic agents. The largest effects were produced by bleomycin. Bleomycin also produced DNA breakage *in vitro* that was easily detected by the alkaline elution technique. The results suggest that a sensitive assay for bleomycin activity in tissues could be developed. DNA breakage is the probable mechanism of bleomycin actions. Support for this contention was derived from concurrent measurements of DNA breakage and cell killing.

Several chemotherapeutic agents were observed to produce smaller extents of DNA breakage. It seemed possible that some of these effects could result indirectly from the action of the enzymatic excision repair system in the cell. This possibility was explored by using xeroderma pigmentosum (XP) fibroblasts which lack excision repair. Of the agents tested, only anthramycin produced less DNA breakage in XP cells than in normal cells, indicating that the excision repair system plays a role in the production of DNA breaks by this agent. Other agents, including bleomycin, BCNU, and actinomycin produced equal breakage in the two types of cells. The finding with anthramycin fits our previous conclusion that this drug binds covalently to DNA. The sites of drug binding could therefore activate the excision process.

The breakage and rejoining of DNA strands during excision repair was studied further by alkaline elution, since previously available techniques had not been adequate to define this aspect of the repair process. The formation and resealing of DNA single-strand breaks after ultraviolet irradiation of normal human fibroblasts occurred as expected according to the current picture of excision repair. Fibroblasts from patients with xeroderma pigmentosum, known to be deficient in DNA repair synthesis, failed to exhibit DNA breaks after UV. Cells of the variant form of XP characterized by normal DNA repair synthesis exhibited normal production of breaks after UV, but were slower than normal cells in resealing these breaks.

The rejoining phase was inhibited by caffeine in XP variant cells, but not in normal cells. Rejoining in normal cells was inhibited by chloroethyl isocyanate, (CIC) a breakdown product of BCNU that we had previously found to inhibit strand rejoining after X-ray.

Another type of DNA lesion relevant to chemotherapy is crosslinking, either between DNA strands or between DNA and protein. The alkaline elution method was found to be adaptable to the measurement of such crosslinks. The effects of nitrogen mustard (HN2), a known crosslinking agent were readily measurable at pharmacologically pertinent concentrations. Ultraviolet irradiation was also found to produce DNA crosslinking which was repaired in normal human fibroblasts but not in repair-deficient XP cells.

We have initiated studies of drug effects on replication meachanisms in mammalian cells. The alkaline elution technique was well suited to studies of the later stages in the growth of newly synthesized DNA strands, when the strand lengths become too long to be readily measured by previous techniques. We are studying the effect of a period of DNA synthesis inhibition on the ability of DNA strands, caught in the process of replication, to resume growth after removal of the inhibitor. By studying the resumption of replication events after removal of inhibitor, we hope to learn what may be responsible for the toxicity of S-phase-specific drugs.

Earlier events in replication, when new DNA chains are still short, can be studied bv sedimentation in alkaline sucrose gradients. We have completed a set of studies in untreated L1210 cells that will serve as a starting point for studies of drug effects.

The earliest events in replication are concerned with the initiation of new DNA strands or replicons. Initiation is suspected to involve the synthesis of an RNA primer segment, but the evidence for this in mammalian cells has been conflicting. We are attempting to make a clear demonstration of such RNA segments. If successful, this will lead to studies of drug effects on these RNA-related processes.

The question of how to selectively kill cancer cells can be turned around to ask what permits the long-term survival and proliferation of transformed cells while normal cells often appear to have a limited capacity for proliferation. Our laboratory is exploring the possible role of selective degradation of proteins. Defective protein molecules may accumulate during senescence of normal cells, and may be responsible for their limited proliferation. Selective degradation of defective proteins, formed by incorporation of amino acid analogues, was demonstrated in a strain of normal human cells. Studies are in progress to compare the selective degradation of defective proteins in normal, senescent, and transformed human cells.

Studies were begun on a potential cell-culture model for chemotherapy. The rationale being tested is that a therapeutic advantage may be achieved by selective protection of normal cells. We assume that neoplastic cells invariably differ from normal cells in that they have defective proliferation control. If this proliferation control could be activated, for example by means of a metabolic inhibitor or hormone, normal cells would stop proliferating whereas neoplastic cells would continue to proliferate. A cycle-specific drug could then be added to selectively kill proliferating neoplastic cells. This rationale was tested in cultures of normal and transformed human fibroblasts using an aminonucleoside analogue of adenosine as the protective agent and hydroxyurea as the killing agents. We have demonstrated that at least a partial protective effect can be achieved, and we are now examining the parameters of the combination drug regimen in order to optimize the effect. A related project to further pursue these ideas using analogues of cyclic nucleotides in mouse cells was initiated in the Laboratory of Molecular Biology, Division of Cancer Biology and Diagnosis.

Project No. Z01 CM 06112-05 LMP
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Effects of Chemotherapeutic Agents on DNA

Previous Serial Number: Same

Principal Investigators: Kurt W. Kohn, Albert J. Fornace, and Leonard C. Erickson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	4.6
Professional:	2.6
Other	2.0

Project Description:

Objectives:

(1) To determine the effects of chemotherapeutic agents on DNA structure and function in cells.

(2) To determine the effects of drugs on DNA replication and repair mechanisms.

(3) To relate the above effects with lethality in various cell types.

Methods Employed:

(1) Cell culture of various cell lines in suspension and surface cultures. Cell lines used include human fibroblasts of various origins and mouse L1210 cells.

(2) Radioactive precursors.

(3) X-ray and UV-irradiation of cells.

(4) Preparative ultracentrifugation: (a) velocity sedimentation in sucrose gradients; (b) equilibrium sedimentation in Cs gradients.

(5) Analytical ultracentrifugation.

(6) Alkaline elution (a new method devised in this laboratory).

Major Findings:

1) Alkaline elution analysis of DNA from mammalian cells.

This technique for measuring DNA single-strand breaks, originally developed in our laboratory, was further studied to determine its basis and its potential range of application. The technique is now the most sensitive available measurement of DNA single-strand breaks in mammalian cells, and is able to quantitate changes in DNA occurring after sub-lethal doses of X-ray and other DNA-damaging agents. The procedure essentially is to lyse cells on filters and then to elute the lysate with alkali. When this is done under proper conditions, the rate of elution of DNA is a highly sensitive measure of DNA single-strand length.

In order to properly interpret results obtained using this method, it is desirable to understand the physical basis for the DNA fractionation obtained. Three factors were considered that might limit the rate of release of DNA single-strands: (1) Incomplete dissociation of hydrogen-bonded base-pairs. The longer the DNA molecule, the greater would be the probability of residual base-pairing that could limit elution. (2) Alkali-stable linkages of DNA to cell structures (for example, nuclear membrane) that do not pass thru the filter. Single-strand breaks would separate DNA segments from the points of attachment and allow these segments to pass thru the filter. (3) Physical entanglement of the strands. The strands would have to become disentangled, in a diffusion-limited process, before they could be eluted. The rate of this process would be highly dependent on strand length.

These possibilities were tested by measurement of the initial kinetics of elution as a function of pH. For these experiments, L1210 cells growing in suspension culture were labeled for 20 hours (the cell doubling time is 12 hrs.) with ^{14}C-thymidine. The cells were then subjected to low doses of X-ray at 0° in order to generate known frequencies of single-strand breaks. The major findings were as follows. (1) As pH is raised, there is a sharp transition from very slow elution to rapid elution. The transition occurred at pH 11.7-11.8. This indicates that strand-separation is required for elution. In support of this, we found that the eluted DNA is almost exclusively single-stranded, as measured by CsCl equilibrium sedimentation after neutralization. (2) Above pH 11.8, the initial elution kinetics become independent of pH. This excludes the possibility that elution is limited by alkali-labile links or hydrogen bonds. (3) The initial elution kinetics accurately obey a first-order relation. This type of kinetic relation could result from a random distribution of DNA single-strand breaks and a simple relation between disentanglement time and strand length. (4) The relation between apparent first-order rate constant for elution and X-ray was linear in the range of 0-300 rad. There was no indication of a dose threshold, such as might be expected if the elution were limited by discrete DNA attachment points. These findings are most easily explained on the basis of disentanglement of single-strands as the rate-limiting step for DNA elution.

Although the factor that limits elution of extremely long strands appears to be disentanglement, the elution of shorter strands can be limited by pH. The range of strand lengths accessible for measurement might be expanded to include shorter strands by manipulation of pH. We explored this possibility by eluting sequentially with a series of solutions of increasing pH. When the cells had received 2,000 rad or more of X-ray, a large fraction of the DNA eluted in discrete steps as the pH was increased. Under these conditions, the elution of the DNA was limited by pH rather than time. Further increase in X-ray dose, increased the portion of the DNA that eluted at lower pH. The lowest pH to give significant elution was pH 11.3.

The above results were all obtained by using tetrapropylammonium as the only cation in the alkaline eluting solution. This had previously been found to give better results than other cations. When a similar set of pH-step experiments were conducted using Na^+ as the cation, it was found that a higher pH range was required to give similar extents of elution. This difference between sodium and tetrapropylammonium cations was also observed using pulse-labeled cells instead of X-ray'd cells.

We conclude that DNA fractionation can be carried out either by time-dependent elution for very long strands or by pH-dependent elution for shorter strands.

2) DNA single-strand breaks during the repair of UV damage in human fibroblasts and the abnormalities of repair in xeroderma pigmentosum.

The first step in the repair of UV damaged DNA in human cells is single-strand scission near the site of damage. The final step is the joining of newly synthesized DNA segment to the end of the pre-existing DNA strand. In the first step, DNA breaks are produced; in the last step, they are removed. These steps have been particularly difficult to study, because of inadequate sensitivity of the ordinary techniques to measure strand breaks. We were able to measure these steps by using the technique of DNA alkaline elution.

The general features of our results were consistent with current concepts of excision repair. Immediately following UV, there are no breaks; but within a few minutes of incubation after UV, the extent of DNA breakage has reached a maximum. This is consistent with the idea that the DNA scission is enzymatic Upon further incubation of the cells after UV, the DNA breaks disappear at a rate strongly dependent on the UV dose. After 15 erg/mm^2 of 254 nm radiation (a sub-lethal dose in normal cells), the repair of break is complete within 1 hr. After 100 erg/mm^2, the time required is 15 hrs. Xeroderma pigmentosum (XP) cells of the sort that are known to lack the capability for DNA repair synthesis do not form DNA breaks after UV. This fits the current idea that these cells may lack a specific UV-endonuclease.

There is a variant form of XP in which the patients have the skin manifestation of the disease, but in which the cells have normal DNA repair synthesis, and the early steps in excision repair are apparently normal. Previous work, however, had not excluded the possibility of a defect in the rejoining step.

We found that these cells do have a measurable defect at this step. The rejoining rate after 100 erg/mm^2 of UV was slower than normal, and the difference from normal was exaggerated by caffeine. Caffeine had been reported to inhibit post-replication repair in these cells. Our experiments were conducted on confluent, non-replicating cells, so that our findings must be related solely to DNA repair. A model was proposed to explain both defects on a common basis. It was proposed that the basic defect is that the cells have lost the ability to cope with UV-damage in the template. The block in rejoining that we observe would be explained on the basis of pairs of UV lesions located near each other but on opposite strands. If one lesion is within the excision range of the other, the DNA repair polymerase which fills the gap would encounter a template lesion and be blocked at that point. This possibility is quantitatively consistent with known estimates of frequency of UV lesions and size of excision gap. The theory predicts that the abnormality should be much more apparent at high UV dose, since the frequency of dose pairs of lesions should increase with the square of the dose. Consistent with this expectation, was the finding that when the dose was reduced to 15 erg/mm^2, the difference from normal nearly vanished.

We had previously found that the rejoining of single-strand breaks after exposure of L1210 cells to X-ray was inhibited by chloroethyl isocyanate (CIC), a breakdown product of BCNU. We now find that CIC also inhibits the rejoining phase of DNA repair following UV, both in normal human fibroblasts and HeLa cells. The parent drug, BCNU, produced less inhibition of rejoining, but had a greater effect than CIC in producing DNA breaks. CIC did not inhibit DNA repair synthesis after UV, indicating that the point of inhibition is probably at the DNA ligation step (the final step in strand rejoining).

3) Crosslinking of DNA in mammalian cells by UV radiation or nitrogen mustard (HN2).

Nitrogen mustard is known to crosslink between opposite strands of the DNA helix. Crosslinks between DNA and protein also are possible. UV radiation also has the potential for these two types of crosslinking, but DNA-protein crosslinking probably is much more prominent than DNA inter-strand crosslinking. Either of these types of crosslinks should affect the alkaline elution of DNA. Crosslinking should diminish the elution induced by a given dose of X-ray. This expectation was fulfilled both by HN2 and by UV. In the case of HN2, a clear separation between crosslinked and uncrosslinked DNA was achieved, both in L1210 cells and in human fibroblasts. Crosslinking was demonstrated after exposure of cells to 10^{-7}M HN2. The sensitivity of measurements thus includes the concentration range of pharmacologic interest.

The crosslinking effect of UV was of immediate interest from two points of view: (1) does crosslinking affect the interpretation of the data on the appearance and resealing of DNA breaks during repair, and (2) are crosslinks themselves repaired? We found that UV causes a crosslinking effect both in normal and xeroderma pigmentosum (XP) fibroblasts. The crosslinking may be due to direct photochemical reaction, since the effect occurred without post-UV incubation. In normal cells, the crosslinking was repaired, but in XP cells crosslinking increased with post-UV incubation. Although, the crosslinking quantitatively affected the data on DNA breakage during repair, it did not alter the qualitative conclusions. Specifically, the difference observed between normal and XP variant cells was not due to differences in crosslinking.

4) Relation between excision repair and chemotherapeutic agents.

We have previously found that a variety of chemotherapeutic agents (agents that have passed Decision Network 2) produced detectable DNA single-strand breakage as measured by alkaline elution. These previous studies were in L1210 cells. We are now asking the question whether any of these effects are produced thru the action of the excision repair system. Evidence for an affirmative answer would be that an agent produces breaks in normal human fibroblasts but not in XP fibroblasts. Clear results have so far been obtained on 4 agents, of which 3 were negative and 1 was positive. Bleomycin, BCNU, and actinomycin produced single-strand breakage equally in normal and XP cells. Anthramycin, however, consistently produced more effect on the DNA in normal cells than in XP cells. This was of special interest since our previous work indicated that anthramycin binds covalently to DNA. Thus the drug could provide a target for excision repair. This raises the question of whether the action of the excision repair system would help or hinder the survival of cells treated with irreversible DNA-binding drugs.

5) DNA damage and cytotoxicity by bleomycin.

Bleomycin is known to be able to produce DNA single-strand breaks. We are studying this process using the alkaline elution technique. Studies were carried out on lysed cells and on intact cells. For the former, untreated L1210 cells were lysed on filters by a detergent-EDTA lysis solution, as in the standard elution procedure. The same solution, containing various concentrations of bleomycin, was then added and kept in contact with the filter, usually for 2 hrs. The standard alkaline elution procedure was then carried out. The bleomycin caused an increase in DNA elution, which depended on bleomycin concentration, exposure time, and pH. It was hoped that this procedure would be sensitive enough to provide a method of measuring bleomycin activity in biological materials. Under optimum conditions, we obtained large effects with concentrations down to 0.1 μg/ml. Given a suitable method for extraction of bleomycin from cells, this could be an adequate sensitivity.

We attempted to enhance sensitivity by adding dithiothreitol (DTT), which has been reported to enhance DNA breakage by bleomycin. In our system, however, DTT was so active in producing DNA breaks by itself that any effect on bleomycin was obscured.

The activity of bleomycin on DNA in the cell lysates had an optimum at pH 8-9. We asked whether bleomycin also exhibited a pH dependence in its effect on intact cells. We found that this was indeed the case. The effects of bleomycin on the growth rate, the survival of clone-forming cells, and the effect on intracellular DNA in L1210 cells all increased with pH in range of 6.5 to 8.0.

6) Drug effects on DNA replication.

The alkaline elution procedure was previously shown to be applicable to studies of the formation of new DNA strands during replication. The procedure is particularly suited to studies of the later stages of this process where very long strands are formed. We are studying the effects of transient inhibition of replication by hydroxyurea on the subsequent course of replication. The question is whether a period of inhibition can cause a prolonged perturbation of the replication process. We have found that DNA that has been synthesized immediately prior to inhibition of synthesis by hydroxyurea (HU) can resume its maturation into long strands when HU is removed several hours later. The rate of conversion to mature strands, however, appears to be reduced. Proper interpretation of results in this system will require studies in synchronized cells.

Studies were also conducted with the view of examining drug effects at earlier steps in chain replication. The alkaline sedimentation technique was perfected for studying events at the stage of short chains.

For studies at the earliest steps of initiation of DNA chains and replicons, we are exploring the possibility of detecting RNA primer segments attached to short DNA chains labeled by very short pulses. This work was initiated at Florida State University by J. H. Taylor, M. Wu, and L. C. Erickson, and is being continued in our laboratory. One of the problems in this work has been the difficulty in obtaining an adequate quantity of presumptive RNA-containing DNA for study. We have made some progress towards solving this problem by using suspension cultures of L1210 cells to facilitate the production of large numbers of highly labeled cells. We were able to demonstrate the transient appearance of DNA material at a buoyant density shifted towards that of RNA. This material was previously seen only when FUdR was used to slow replication and increase labeling density. In L1210 cells, we were able to detect this material without using drugs.

Proposed Course:

(1) Further development of alkaline elution procedures.

a. Determine the role of cell protein on DNA elution. Is there linkage between DNA and protein? Do crosslinking agents increase such linkage? Can DNA-protein crosslinks be distinguished from DNA inter-strand crosslinks?

b. Devise a pH-gradient elution procedure for fractionation of a broad range of DNA strand lengths.

c. Develop a method for determination of bleomycin activity in cells and tissue.

(2) Study effects of bifunctional alkylating agents on DNA in various cell types.

a. Do all types of bifunctional alkylating agents produce crosslinks? Are there differences in regard to DNA inter-strand crosslinks versus DNA-protein links?

b. Are crosslinks repaired? If so, is it by means of the excision repair system -- e.g., are xeroderma pigmentosum (XP) cells able to carry it out?

c. Can differences in crosslinking and repair be discerned between cell lines of different sensitivities to the drugs?

d. Do other drugs affect the formation and repair of crosslinks by alkylating agents? Can this be a basis for drug combination?

(3) Study effects of DNA synthesis inhibitors on the course of DNA replication after removal of the inhibitors. Do this in synchronized cells. Compare the fate of DNA synthesized immediately prior to addition of inhibitor with that synthesized immediately following removal of inhibitor. If our attempts to demonstrate RNA primer segments at the initiation ends of newly synthesized DNA chains or replicons are successful, we will be able to look for specific drug effects on initiation events.

(4) Study effects of bleomycin on various cell types. Can survival be related to extent of DNA breakage? Can a second drug affect the extent of DNA breakage caused by bleomycin, and would this correlate with cell survival?

Keyword Descriptors:

DNA damage
DNA repair
replication
DNA single-strand length
DNA crosslinking
alkaline elution
density gradient centrifugation
cell culture
xeroderma pigmentosum
X-ray
ultraviolet radiation
chemotherapeutic agents
bleomycin
alkylating agents
BCNU

Honors and Awards: None

Publications:

Kann, H.E., Jr., Kohn, K.W., Widerlite, L., and Gullion, D.: Effects of 1,3-bis(2-chloroethyl)-1-nitrosourea and related compounds on nuclear RNA metabolism. Cancer Research 34: 1982-1988, 1974.

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Kohn, K.W.: Anthramycin. In Corcoran, J.W., and Hahn, F.E. (Ed.): Antibiotics. Springer-Verlag, 1974, pp. 1-11.

Kohn, K.W., Friedman, C.A., Ewig, R.A.G., and Iqbal, Z.: DNA chain growth during replication of asynchronous L1210 cells. Alkaline elution of large DNA segments from cells lysed on filters. Biochemistry 13: 4134-4139, 1974.

Kohn, K.W., Waring, M.J., Glaubiger, D., and Friedman, C.A.: Intercalative binding of ellipticine to DNA. Cancer Research 35: 71-76, 1975.

Iqbal, Z.M., and Kohn, K.W.: Ribonuclease-resistance patterns of RNA from L1210 cells. Arch. Biochem. Biophys. 166: 518-524, 1975.

Project No. Z01 CM 06130-01 LMP
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Production and Repair of Macromolecular Errors and Damage

Previous Serial Number: None

Principal Investigators: Matthews O. Bradley and Kurt W. Kohn

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.2
Professional:	0.6
Other:	0.6

Project Description:

Objectives:

We are studying the production, repair, and degradation of macromolecular errors or damage produced within cells either spontaneously during information transfer and metabolism or by exogenous physical and chemical agents. In particular we are asking the following questions: Does the protein degradative system act as an error-correcting system by preferentially degrading abnormal proteins? What are the relative capacities of normal, senescent, and transformed cells to selectively degrade abnormal proteins? What is the rate of error generation during the synthesis of collagen in young, transformed, and senescent human fibroblasts? Do amino acid, RNA, and DNA base analogs increase translational errors in collagen? What effects do these and other chemotherapeutic drugs have on mutation rates, on the fidelity of DNA replication and repair, and on RNA and protein turnover? What are the capacities of various cells to repair X-ray damage to DNA?

Methods Employed:

1) Pulse-chase and approach to equilibrium measurements of protein degradation.

2) Isolation, characterization, and collagenase digestion of human fibroblast collagen *in vitro*.

3) Measurements of DNA repair by alkaline elution from filters.

4) Cell culture and cloning methods using WI-38, VA-13, and CHO cells.

5) Gel electrophoresis and column chromatography of proteins.

6) Analysis of drug resistant mutants of CHO and WI-38 fibroblasts.

7) Inverted phase photomicroscopy.

Major Findings:

1) Capacity of normal, transformed, and senescent human fibroblasts to degrade abnormal proteins.

The principal investigator had previously reported that human fibroblasts selectively degrade altered proteins (i.e. those substituted with amino acid analogs). This finding led to the postulate that protein degradation may function, in part, to maintain a low, steady-state protein error frequency and that increased levels of abnormal protein may result from functional failures in protein degradation. In the last few months, experiments begun at Stanford University have been repeated here and demonstrate a quantitative deficit in the ability of senescent fibroblasts to degrade protein that has been altered by canavanine substitution.

2) DNA repair after X-irradiation in normal, senescent, and progeroid human fibroblasts.

Other laboratories have reported that senescent and progeric human fibroblasts are deficient in the repair of X-ray induced single-strand DNA breaks as measured by alkaline sucrose gradients and by unscheduled DNA synthesis. We have repeated these experiments using the alkaline elution method developed previously in this laboratory. To date, all senescent and progeric fibroblasts that we have tested repair X-ray damage as efficiently as do normal fibroblasts.

3) Determination of tryptophan substitution into collagen:

Mature collagen α chains lack tryptophan and represent 8% of the total protein synthesized by WI-38 cells. Collagen is one of the few proteins that is soluble in 0.5 N acetic acid and is the only protein digested by purified collagenase. For these reasons collagen is an ideal protein for detecting errors of protein synthesis, since one can monitor misincorporation of radioactive tryptophan into collagen. The principal investigator has shown previously that addition of ^{14}C- and ^{3}H-tryptophan to pre-confluent cultures of WI-38, results in the incorporation of radioactivity into material, presumably collagen, that is acetic acid soluble and collagenase digestible. These experiments are being continued here in order to demonstrate more conclusively that tryptophan has been incorporated into collagen α chains and to determine the frequency of misincorporation in various cells and during different drug treatments.

Proposed Course:

1) To determine the spontaneous frequency of tryptophan misincorporation into α-collagen of WI-38 cells; to determine whether amino acid and RNA base analogs increase this frequency.

2) To further characterize protein degradation in normal and transformed human fibroblasts under a variety of growth conditions and drug treatments.

3) To determine whether DNA repair is normal in senescent and progeric human fibroblasts.

4) To determine whether amino acid and RNA base analogs are mutagenic in CHO cells.

Keyword Descriptors:

DNA repair
protein degradation
fibroblasts
collagen
protein synthesis
errors

Honors and Awards: None

Publications: None

Project No. Z01 CM 06131-01 LMP
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: *In Vitro* Models for Chemotherapy in Human Cells

Previous Serial Number: None

Principal Investigators: Matthews O. Bradley and Kurt W. Kohn

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.3
Professional:	0.6
Other:	0.7

Project Description:

Objectives:

To develop an *in vitro* system, utilizing normal and malignant human cells, for testing various chemotherapeutic strategems; to utilize this system for understanding the differences in proliferation control between normal and malignant human cells; to test the rationale that reversibly arresting the proliferation of normal cells while allowing continued proliferation of malignant cells, will yield a chemotherapeutic advantage; to find drugs that can be utilized in this way.

Methods Employed:

1) Monolayer culture of WI-38, VA-13 and HeLa cells.

2) Incorporation studies of radioactive drugs and DNA, RNA, and protein precursors.

3) Inverted phase photomicroscopy.

Major Findings:

1) Confluence as a selective blocker of normal cell proliferation.

Confluent, WI-38 cultures contain 2-3 layers of cells and are essentially non-mitotic, being arrested in G1. VA-13 cells, however, proliferate to approximately 3 times the density of WI-38 and will continue to proliferate when seeded on top of a confluent culture of WI-38. If hydroxyurea is added to such a mixed culture, the normal WI-38 cells remain and proliferate after hydroxyurea is removed and the cells are subcultured. We are not yet certain that all the transformed cells can be killed, although no transformed clones have arisen from mixed cultures treated as described. This result demonstrates that normal cells can be protected from hydroxyurea if they are kept non-proliferating, while malignant cells that continue to proliferate will be killed.

2) Aminonucleoside of puromycin as a selective blocker of WI-38 proliferation.

Literature reports indicated that puromycin aminonucleoside should reversibly block the proliferation of WI-38 cells while allowing the continued proliferation of VA-13 cells. We have confirmed that this report is accurate, and have used this drug to protect normal human cells from being killed by hydroxyurea. To date our results show that under carefully controlled conditions proliferating WI-38 cultures that have been treated with both aminonucleoside and hydroxyurea will survive and proliferate again as either clones or mass populations. However, WI-38 cells that have been treated with only hydroxyurea are irreversibly damaged and will neither clone nor proliferate. VA-13 cells are killed if they are treated with either hydroxyurea alone or with both drugs together.

These results suggest that in vivo chemotherapy could be made more effective by using S-phase agents in combination with drugs that reversibly inhibit normal proliferating bone marrow and gastrointestinal epithelia.

Proposed Course:

1) To more carefully define the parameters controlling protection of WI-38 cells from hydroxyurea by aminonucleoside. Specifically, we will define the effects of different treatment times and aminonucleoside concentrations on DNA, RNA and protein synthesis in both normal WI-38 and transformed VA-13 cells. We will also examine the incorporation and loss of radioactive aminonucleoside into VA-13 and WI-38 cells. Using these findings, we will design an optimized regimen for selectively killing transformed VA-13 cells in mixed cultures of normal and transformed human cells.

2) To utilize this system to better understand in vitro growth control of normal and neoplastic human cells. Specifically, we will examine alterations in protein degradation and precursor incorporations during growth transitions in normal and transformed human cells. We will also attempt to differentially inhibit VA-13 and WI-38 cells from entering S-phase by the addition of selected inhibitors of macromolecular synthesis.

Keyword Descriptors:

fibroblasts
chemotherapy
transformation
hydroxyurea
puromycin aminonucleoside

Honors and Awards: None

Publications: None

SUMMARY REPORT

LABORATORY OF TUMOR CELL BIOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1974 -- June 30, 1975

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation *in vitro*. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in *in vitro* and *in vivo* systems. This is the ultimate goal of the Laboratory.

During the past year a number of findings were reported by investigators from the Laboratory:

1. Demonstration that many cases of human myelogenous leukemia contain reverse transcriptase which is immunologically related to reverse transcriptase from tumorigenic subhuman primate type-C viruses.

2. Demonstration that chronic lymphocytic leukemia cells contain a reverse transcriptase-like enzyme which is not related to reverse transcriptase from known RNA tumor viruses.

3. Demonstration that the intracellular form of reverse transcriptase in type-C virus infected cells, as well as, in human leukemic cells may exist in two forms and may be associated with other cellular factors which may affect the process of reverse transcription.

4. Demonstration that placenta from rhesus monkeys contains reverse transcriptase which is immunologically related to reverse transcriptase from endogenous type-C virus from baboon placenta but not to reverse transcriptase from tumorigenic subhuman primate viruses.

5. Demonstration that cells from some patients with myelogenous leukemia contain deoxynucleotidyl transferase, an enzyme formerly thought restricted to lymphocytic cells of thymic origin.

6. Preparation of antisera to DNA polymerase I from normal lymphocytes, and the demonstration that this antisera does not inhibit other normal cellular DNA polymerases or reverse transcriptae from leukemic cells or RNA tumor viruses.

7. Purification and characterization of ribonuclease H from human leukemic cells and the demonstration that this enzyme is not associated with reverse transcriptase (as is the case for reverse transcriptase from avian myeloblastosis virus).

8. Demonstration that ribonuclease H is not associated with reverse transcriptase in mammalian type-C viruses.

9. Purification and characterization of DNA polymerase III (R-DNA polymerase) from normal and leukemic human leukocytes.

10. Development of a procedure for the rapid separation and purification of all known DNA polymerases from mammalian cells.

11. Development of a procedure for the identification of reverse transcriptase in small quantities of leukemic cells (< 1 gm).

12. Purification of DNA polymerses from human leukocytes and several RNA tumor viruses for antisera preparation (in progress).

13. Development of a method of enzyme isolation and of an assay system which permits purified reverse transcriptase from type-C mammalian viruses to transcribe heteropolymeric RNA efficiently in reconstituted reactions.

14. Demonstration that under unusual reaction conditions *E. Coli* DNA polymerase can transcribe heteropolymeric RNA.

15. Demonstration that the endogenous DNA polymerase reaction in the microsomal fraction of human leukemic cells is both RNA-primed and RNA-directed, whereas this reaction in normal human lymphocytes is RNA-primed but DNA-directed.

16. Development of a synchronized endogenous DNA polymerase reaction in RNA-tumor viruses which may permit the sequence analysis of DNA synthesized on viral RNA.

17. Identification and characterization of ribonucleotide terminal transferase in murine type-C RNA viruses.

18. Induction of the extracellular production of type-A particles (which have previously only been reported intracellularly) and demonstration that these particles are immunologically and biologically related to type-C leukemia viruses.

19. Elucidation of the pharmacological effects of cordycepin (a post-transcriptional inhibitor) and dexamethasone (apparently a translational stimulator) on RNA tumor virus production by cells.

20. Demonstration that rifamycin SV derivatives (which were previously reported to inhibit the *in vitro* and *in vivo* activity of RNA tumor viruses after direct incubation) increased the latent period to the development of leukemia in AKR mice when administered to pregnant and to newborn mice.

21. Demonstration that the administration of cordycepin to murine leukemia virus (Rauscher) infected mice inhibited the degree of splenomegaly (50%) but did not inhibit the incidence of leukemia.

22. Demonstration and characterization of two types of leukocyte colony stimulating factor in medium conditioned by human peripheral blood leukocytes.

23. Demonstration that media conditioned by selected fibroblastic culture cells from human embryos markedly stimulate the growth and differentiation of myelogenous leukemia cells in liquid suspension culture.

24. Purification and characterization of colony stimulating factors from human lymphocytes and development of specific antisera (in progress).

25. Demonstration that the endogenous, RNA-directed DNA polymerizing complex in the microsomal fraction of human leukemic cells can be manipulated by gel chromatography and by isopycnic and velocity gradient centrifugation in a similar fashion to RNA tumor virus particles.

26. Demonstration that the genomic RNA of RNA tumor viruses are related to one another in the same manner as the phylogenetic relationship of their respective homologous host cells; this suggests that an RNA tumor virus evolves from the cellular genome.

27. Demonstration that endogenous (usually nononcogenic) RNA tumor viruses (Class I) are highly related to DNA of the progenitor cell, whereas another group (Class II) (oncogenic) are much less related to DNA from the progenitor cell.

28. Demonstration that infection of a cell with a RNA tumor virus results in marked alteration of the genome of the emerging virus.

29. Partial elucidation of the composition of RNA tumor virus genomic RNA by molecular hybridiztaion and sequence analysis.

30. Demonstration that DNA synthesized by the endogenous DNA polymerase reaction complex of human myelogenous leukemia cells has extensive sequence homology to genomic RNA from primate type-C virus; additionally, there may be sarcoma-specific sequences present in myelogenous leukemia cells.

31. Demonstration that the genomic RNA of selected RNA tumor viruses (subhuman primate; sarcoma-specific) is related to cytoplasmic DNA but not to nuclear DNA from human leukemic cells.

32. Development of microassays for the in situ detection of virus-specific nucleic acids (molecular hybridization) or proteins (antibody assays) (in progress).

Z01 CM 06117-04 LTCB
1. Laboratory of Tumor Cell Biology
2. Section on Cellular Control
   Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Mechanisms Leading to Neoplastic Transformation; On Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and On the Development of Biochemical Markers of Neoplastic Cells.

Previous Serial Number: NCI-6117

Principal Investigator: Robert C. Gallo, M.D.

Other Investigators: Major Senior Colleagues: Robert Gallagher, R. Graham Smith, Prem Sarin, David Gillespie, W. Carl Saxinger. Other collaborators and associates: Jerome Donlon, Michael Paran, Gurmit Aulakh, Flossie Wong-Staal, H. Mondal, Hartmut Schetters, Richard Adamson, Lawrence Loeb, George Todaro, Jeff Schlom, Umberto Torelli, Leon Dmochowski, Sol Spiegelman, Robert Nowinski, Robert Ting, Ken Takemoto, Paul Peebles, William Feller, Rolf Neth, Peter Reichard, Natalie Teich, Robin Weiss, Michael Hanna, James Ihle, Fritz Deinhardt, Wolfe Prensky, Ray Gilden, Stu Aaronson, Ken Smith, Dani Bolognesi, B. Lozzio, Ken McCredie, and Marcel Baluda.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI; The Institute for Cancer Research; Viral Leukemia and Lymphoma Branch, NCI; Breast Cancer Segment, NCI; University of Modena; M. D. Anderson Hospital and Tumor Institute; Columbia University College of Physicians and Surgeons; McArdle Laboratories; Biotech Laboratories; National Institute of Allergy and Infectious Disease; Viral Leukemia and Lymphoma Branch, NCI; Georgetown University; University of Hamburg; Karolinska Nobel Institute, Sweden; Imperial Cancer Research Fund, London; Frederick Cancer Research Center; University of Illinois; Sloan-Kettering Institute; Flow Laboratories; Viral Carcinogenesis Branch, NCI; Dartmouth Medical School; Duke University; University of Tennessee; University of California at Los Angeles.

Man Years:

Total: 22
Professional: 13
Other: 9

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

    a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

    b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformation?

    c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell as in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up following the techniques of Paran and Sachs (Paran has recently left our laboratory). Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

5. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

6. Isolation and characterization of type-C virus which was produced by leukemic myeloblasts grown in liquid suspension (see above) and the development of biological and biochemical markers for human leukemia.

7. Helping to elucidate the mechanisms involved in the initiation of DNA synthesis in normal and neoplastic cells and the development of potent and selective DNA polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

8. Markers: (a) an immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (*J. Clin. Invest.* 48: 105-116, 1969; *Science* 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; *Blood* 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (*Nature New Biology* 240: 67-72, 1972; *Proc. Natl. Acad. Sci.* 69: 2879-2884, 1972; *Proc. Natl. Acad. Sci.* 69: 3228-3232, 1972; DNA Synthesis *in vitro*, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (*Nature* 234: 194-198, 1971; *J. Virol.* 12: 431-439, 1973).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (*J. Natl. Cancer Inst.* 46: 789-795, 1971; *Science* 165: 400-402, 1969).

5. Product analysis of DNA synthesized by various DNA polymerases is carried out by conventional techniques including: (a) "Nearest neighbor" analysis; (b) Size estimate and nature (DNA-RNA or DNA) were made by gel electrophoresis and by glycerol gradient ultracentrifugation untreated, heat-treated, RNase and/or alkali treated.

6. *In vitro* leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture, as recently reported (*Science* 187: 350, 1975).

7. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

8. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (*Proc. Natl. Acad. Sci.* 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (d) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

9. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (XC test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

10. Virus quantiation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

Major Findings

1. Identification and partial characterization of a conditioned media factor produced by human embryonic culture cells which sustains exponential growth of myeloid leukocytes from patients with myelogenous leukemia in liquid suspension culture.

2. Growth and partial characterization of myeloid leukocytes in liquid suspension of cells from 16 patients with acute or chronic myelogenous leukemia.

3. Identification of a type-C virus produced by one of the cultured human myelogenous leukemia cell lines. Virus isolated from three sublines of a blood sample and later again isolated from a bone marrow sample from the same patient.

4. Demonstration that the virus released from human AML cells is infectious for various homologous and heterologous fibroblastic cell culture lines. Demonstration that this virus can rescue integrated murine sarcoma virus. Adaptation of an in vitro test (XC test) to standardize infectious units of virus activity.

5. Characterization of reverse transcriptase from all the primary isolates and the secondary isolates of the human AML type-C virus. Determination that it is immunologically-related to reverse transcriptase from the two known tumorigenic subhuman primate type-C viruses, gibbon ape leukemia virus and woolly monkey sarcoma virus.

6. Demonstration that the intracellular form of viral-related reverse transcriptase from human AML cells and from type-C virus-producing animal cells may be present in an aggregated form which affects the primer-template and immunological properties of the enzyme.

7. Purification and characterization of DNA polymerase γ from several cases of myelogenous leukemia, histiocytic leukemia and myeloproliferative diseases which apparently lack viral-related reverse transcriptase.

8. Demonstration that infection of leukemic leukocytes but not normal leukocytes by non-transforming animal type-C viruses may rescue fibroblast transforming activity from the infected leukocytes.

9. Development of a sensitive in situ radioimmunoassay test system for viral proteins in virus-infected lymphoblastoid cells. Studies are in progress to determine if this test has adequate specificity to use as a diagnostic test with human bone marrow.

10. Carbopol (a Carboxypolymethylene derivative) was found to inhibit the RNA dependent DNA polymerases from RNA tumor viruses (avian myeloblastosis virus, Rauscher leukemia virus or simian sarcoma virus). This compound was shown to inhibit the viral DNA polymerase activity by chelating the divalent cation present in the reaction.

11. A nuclease was isolated and purified from pooled human breast milk. The enzyme has a molecular weight of 24,000 and appears to be specific for poly(C) with low degradative activity for transfer RNA. This nuclease degrades poly(C) by exonucleolytic attack.

12. Type A particles were isolated from MOPC-104C or Leydig cell tumors or N18 neuroblastoma cell lines. Examination of DNA polymerase associated with these particles showed that the enzyme had properties characteristic of cellular DNA polymerase.

13. A poly A polymerase has been isolated and characterized from MOPC-104E tumor cells. The enzyme has a molecular weight of 60,000 and efficiently utilized $(A)_{11-19}$ as the initiator.

14. An attempt was made to isolate and characterize RNA dependent DNA polymerase from 3 sources of murine Xenotropic viruses. NZB mice spleens or NIH swiss mouse radiation lymphoma tissue contained very low levels of viral DNA polymerase Hartley cell line (34889) was found to be good source for the viral DNA polymerase. This cell line also contains terminal deoxynucleotidyl transferase, and attempts are in progress to separate the RNA dependent DNA polymerase from terminal transferase.

15. Attempts to isolate type-C virus from human breast tumor cell line (MCF-7) and to characterize RNA dependent DNA polymerase from these cells, were unsuccessful. These results are in conflict with those of Rich _et.al._

16. Terminal deoxynucleotidyl transferase, an enzyme considered to be specific for thymus gland, was found in cells from some CML patients in acute blast phase of the disease. The presence of high levels of this enzyme in CML patients in acute blast phase of the disease represents a lymphoblastic conversion in CML. The detection of this enzyme in CML patients may be important in deciding the type of treatment to be given to these patients.

17. RNA dependent DNA polymerase from gibbon ape leukemia virus, baboon virus and feline leukemia virus has been purified and characterized. These enzymes are being used for the preparation of antisera.

18. An RNA dependent DNA polymerase was isolated from early gestational rhesus monkey placenta. This enzyme immunologically related to baboon virus DNA polymerase suggesting that other old world primates also harbour endogenous viruses related to the baboon endogenous virus. Attempts to characterize RNA dependent DNA polymerase from human placentas have so far been unsuccessful, mainly because of the difficulty in procuring early gestational samples.

19. Antiserum was prepared in rats against DNA polymerase α isolated from human cells. This antiserum has been found to be highly specific and does not cross react with DNA polymerase β or γ or RNA dependent DNA polymerase.

20. Antisera against RNA dependent DNA polymerases from simian sarcoma virus, avian myeloblastosis virus, Rauscher murine leukemia virus, RD-114 virus and gibbon ape leukemia virus have been prepared. These antisera have been useful as diagnostic probes in studying enzymes from human leukemic cells.

21. Demonstration of the need for more sensitive technique for assay of the presence and role of reverse transcriptase in human leukemic cells by comparative evaluation of conventional methods.

22. Demonstration of the presence of an enzyme which resembles (but may not be identical to) DNA polymerase γ in human blood platelets from normal donors. The similarity of biochemical properties and cellular location (cytoplasmic) to reverse transcriptase from some human leukemic cells suggests a possible precursor relation.

23. Complete characterization of one form of RNase H from human leukemic cells.

24. Demonstration of a second form of RNase H and its characterization (in progress).

25. Particles with biophysical and biochemical properties of complete RNA-tumor viruses have been cytoplasmically located in some cases of human leukemia. These similarities include comparable buoyant densities and sedimentation velocities in sucrose and relatedness to viral reverse transcriptase, structural protein and nucleic acids.

26. Demonstration that DNA products synthesized by the components of the high density cytoplasmic particles from leukocytes of various leukemic and normal patients contain sequences related to a collection of different RNA tumor viruses; but differences are seen in the extent and pattern of hybridization - differences between normal and leukemic patients and between patients with different forms of the disease.

27. Demonstration that the genomic RNA of RNA tumor viruses are related to one another in the same manner as the phylogenetic relationship of their respective homologous host cells; this suggests that an RNA tumor virus evolves from the host genome.

28. Demonstration that the human virus-like genetic information and the primate leukemia virus information have had similar evolutionary histories by comparison with the above pattern.

29. Demonstration that endogenous (usually nononcogenic) RNA tumor viruses (Class I) are highly related to DNA of the progenitor cell; whereas another group (Class II) though much less related to DNA from the progenitor cell could still have arisen entirely from the host cell genome.

30. Demonstration that infection of a cell with an RNA tumor virus results in marked alteration of the genome of the emrging virus.

31. Development of a procedure for measurement and analysis of nucleic acid homologies which provides a more rigorous analysis of the qualitative and quantitative parameters of this reaction and may aid in the designation of the species of origin of RNA tumor viruses.

32. Development of rapid procedures for the identification and purification of viral specific RNA from intracellular and cell free supernatants.

33. Development of a procedure for the simultaneous determination of the dimensions and associated biochemical properties of subcellular particles $\leqq$ 3000 Å. Application of this procedure should allow the morphological identification of subviral cellular intermediates.

34. Development of an efficient sensitive method for the purification and detection of intracellular reverse transcriptase from $\leqq$ 0.1 grams of leukemic cells, using affinity chromatography.

35. Demonstration in a mammalian system that in the endogenous viral DNA synthesis, the primer is a unique species of 4S RNA terminating with 3'-adenosine. Attached to the primer a unique oligodeoxynucleotide sequence of 8-10 residues has been isolated and its sequence partially characterized.

36. Demonstration and characterization of a ribonucleotide terminal transferase present in murine type-C RNA tumor viruses.

37. Elucidation of the life cycle of RNA tumor viruses following their induction in cells transformed by but not producing virus. In various stages of virus infection (transformation, induction by IdU, inhibition with cordycepin, or stimulation with dexamethasone revertant) the amount of integrated viral DNA, expression of viral RNA and protein, and the production of released virus attain characteristic and discernible levels as measured by nucleic acid hybridization, immunoassay and enzymatic activity.

38. Demonstration that cytoplasmic particulate fraction from a mouse plasma tumor (MOPC-104E) which copurified with intrasternal A particles was able to synthesize a poly A sequence 150 nucleotides long using 35S RNA as a primer.

39. Development of a transfection system in which transformation of WHE cells was achieved by incubation in the presence of DNA from human leukemic cells.

40. Achievement of partial purification and characterization of CSF from both adherent cells and nonadherent cells of human peripheral blood in culture. CSF from the nonadherent cells can only stimulate colony formation of primate marrow cells.

41. CSF from murine fibroblasts have been purified extensively and antibodies to the purified CSF have been prepared.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Keynote Descriptors:

Conditioned media factor, type-C virus, cultured human myelogenous leukemia cell lines, XC test, reverse transcriptase, human AML type-C virus, primate type-C viruses, DNA polymerase γ, non-transforming animal type-C viruses, fibroblast transforming activity, radioimmunoassay test, viral proteins, nuclease, type A particles, MOPC-104E, Leydig cell tumors, N18 neuroblastoma cell lines, poly A polymerase, Xenotropic viruses, Hartley cell line (34889), terminal deoxynucleotidyl transferase, human breast tumor cell line (MCF-7), CML, gibbon ape leukemia virus, baboon virus, feline leukemia virus, gestational rhesus monkey placenta, endogenous viruses, baboon endogenous virus, DNA polymerase α, DNA polymerase β, antisera against RNA dependent DNA polymerases, simian sarcoma virus, avian myeloblastosis virus, Rauscher murine leukemia virus, RD114 virus, diagnostic probes, DNA polymerase γ, blood platelets, RNase H, DNA products, high density cytoplasmic particles from leukocytes of various leukemic patients; genomic RNA, phylogenetic relationship, evolutionary histories, viral specific RNA, morphological identification of subviral cellular intermediates, affinity chromatography, 4S RNA, oligodeoxynucleotide sequence, host modification, origin of RNA tumor viruses, life cycle of RNA tumor viruses, transformation, induction by IdU, cordycepin, dexamethasone, integrated viral DNA, expression of viral RNA, nucleic acid hybridization, immunoassay, enzymatic activity, RNA as a primer, transfection, and growth and differentiation factors.

Honors and Awards:

1. Dr. Robert Gallo was the recipient of the Dameshek Award for research in hematology--awarded by the American Society of Hematology, December, 1974.

2. Dr. Robert Gallo was awarded an Honorary Doctor of Science degree (for research in Leukemia); Providence College.

3. Keynote lecture for Edwin R. Kretschmer Memorial Symposium, University of Chicago, February, 1975.

4. Guest lectureships at the following international meetings:

   a. International Congress of Hematology, Jerusalem, Israel, September, 1974.

   b. Munich Tumor Virus Meeting, Munich, Germany, October, 1974.

   c. XIth International Cancer Congress, Florence, Italy, October, 1974.

   d. Symposium on Molecular Basis of Malignancy, Vienna, Austria, October, 1974.

   e. Markers in Cell Differentiation - Evidence for Diagnostic and Therapeutic Trends on Human Leukemia, Wilsede, Germany, June, 1975.

5. Guest lectureships at the following national meetings and universities:

   a. Characteristics of Oncorna and Related Viruses, Washington, D. C., September, 1974.

   b. George Washington University, Department of Genetics, Washington, D.C., November, 1974.

   c. New York University, School of Medicine, Hematology Department, New York, New York, November, 1974.

   d. Dartmouth Medical School, Hanover, New Hampshire, December, 1974.

   e. University of California, San Diego, December, 1974.

   f. University of California, Fresno, California, December, 1974.

   g. California State University, Fullerton, California, December, 1974.

   h. The Salk Institute, San Diego, California, December, 1974.

   i. University of California, School of Medicine, Los Angeles, California, December, 1974.

   j. American Association of Cancer Institutes, Bethesda, Maryland, January, 1975.

   k. University of Kentucky, Biochemistry Department, February, 1975.

   l. Yale University, Department of Pharmacology and Medicine, February, 1975.

   m. University of Tennessee, Knoxville, Tennessee, March, 1975.

   n. University of Pittsburgh, Biochemistry Department, Pittsburgh, Pennsylvania, April, 1975.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR MEDICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1974 - June 30, 1975

The Medical Oncology Area is primarily concerned with clinical and laboratory research related to the treatment of malignant disease. The program embraces the broad area of clinical pharmacology, clinical trials, supportive care, marrow transplantation, and basic virology research. The staff is composed of those who spend most of their time in laboratory research as well as those with a strong primary clinical interest. Clinical research programs are primarily disease-oriented with in-depth investigations of the human biology as well as therapeutic research in these disorders. The entire program is supported by a statistical unit and extensive platelet and leukocyte transfusion support. The program will soon expand its efforts in the use of the protective environments to enhance patient tolerance to intensive treatment programs. The clinical units maintain a close working liaison with a number of other departments at NIH including Pathology, Nuclear Medicine, Pharmacology, and Clinical Pathology.

The entire area has been strengthened by the recent inclusion of the Surgery and Radiation Therapy Branches to the Division of Cancer Treatment. The further application of multimodality therapeutic programs will be enhanced as well as the general educational thrust of the area. Active participation in senior medical student Oncology-Hematology elective program will be continued. The prospect of more junior pediatric staff will strengthen the Pediatric Oncology Branch's program.

Program Accomplishments

Biostatistical Information Systems Unit

The objective of the BISU is the utilization of statistical methodology and computer research technology to maximize knowledge obtained from clinical trials and other research carried out within and on contract to the clinical programs of the Division of Cancer Treatment. The unit participates directly in the design and analysis of clinical trials and other experiments, develops data collection procedures and related computer systems, and maintains computer files of collected data. The unit also conducts research in statistical methodology in support of the above goals.

Marrow Culture Studies

The tissue culture (marrow) laboratory has continued to provide a collaborative resource for other units in the program as well as maintaining independent clinical research efforts. One of the more interesting studies has been in Felty's syndrome. A controversy has existed whether the neutropenia observed in Felty's syndrome is due to hypersplenism alone. It was observed that complement inactivated serum from rheumatoid arthritis patients with this syndrome inhibit autologous in vitro marrow proliferation whereas normal serum does not inhibit normal autologous marrow. This inhibitory factor appears to reside in the IgG component of serum.

Cytogenetic Oncology

This section has continued to provide a tremendous service to the clinical program. Cytogenetic studies (including 500-600 bone marrow and 300 peripheral blood specimens a year) in human neoplastic, hematological, and congenital diseases with special emphasis on: (a) leukemia patients who previously received large dose or total body irradiation for neoplasm; (b) patients with Sezary syndrome; and (c) patients with an initial diagnosis of myelofibrosis with myeloid metaplasia.

Bone marrow cytogenetic studies were performed on 30 patients (10 LSA; 10 Hodgkin's; 2 CLL, and 8 miscellaneous) who developed leukemia after receiving large doses of irradiation for previous malignancies. Up to 80% of these patients had aneuploid cells in all or some of their marrow cells. Four of these 30 patients had Ph' chromosomes. This high incidence of Ph' positive cases in irradiated patients is quite significant and is more than coincidental.

Medicine Branch - Clinical Trials

Phase I, II Investigations

Gallium nitrate is currently under investigation. Hexamethylmelamine, Bleomycin, and Cytembena are in Phase II trial especially in ovarian cancer.

Disease-oriented Studies

Histiocytic lymphoma. Long term results of the intensive chemotherapy of this highly resistant malignancy with MOPP and C-MOPP have been published this year and indicate that approximately 40% of patients with advanced disease will enter a complete remission and most of those completely responding will remain free of disease on no further therapy for periods of up to 9 years. These long-term follow up findings add histiocytic lymphoma to the group of malignancies in which long term disease irradiation with chemotherapy is possible.

Lymphocytic lymphoma. The randomized trial between total body irradiation and chemotherapy in the non-Hodgkin's lymphomas has been completed for most histologic groups and has been submitted for publication this year. The ongoing trial now compares combination chemotherapy with total body irradiation plus appropriate chemotherapy with CVP or C-MOPP.

Ovarian Carcinoma. A large scale study of ovarian cancer, the commonest fatal gynecologic malignancy, has been underway for 3 years combining in-house, contract, and laboratory based investigations. A randomized prospective trial comparing melphalan with a 4 drug combination (Hexa-CAF), including hexamethylmelamine, cyclophosphamide, methotrexate and 5-fluorouracil continues. Preliminary results indicate an overall response rate for the combination of 85% (31% CR) compared to 60% (20% CR) for melphalan.

Osteogenic sarcoma. A prospective adjuvant chemotherapy trial is underway comparing high-dose methotrexate with high-dose methotrexate and adriamycin in patients after primary surgery. In conjunction with the clinical trial, detailed pharmacokinetic studies of high-dose methotrexate are being completed in conjunction with the Laboratory of Clinical Pharmacology.

Breast carcinoma. In addition to the studies summarized under Clinical Program in Breast Carcinoma, follow-up studies on a CMF-(P) regimen previously reported by the Medicine Branch has been completed on 40 patients. The overall response rate is 68% with 20% complete remissions. The median duration of initial remission is 8 months.

American Burkitt's lymphoma. A clinicopathologic study of 30 cases of American Burkitt's lymphoma has been reported this year, with analysis of the important clinical features predicting prolonged survival after chemotherapy. In addition, an analysis of the sites of relapse and pathologic correlations has been published.

Melanoma. A trial comparing adjuvant immunotherapy or chemotherapy to no further therapy after complete resection on Stage II melanoma is underway in conjunction with the Surgery and Immunotherapy Branches, NCI. The immunotherapy regimen employs BCG and autologous-cultured-neurominidase treated melanoma cells. The chemotherapy adjuvant is MeCCNU.

Clinical Investigations

Staging in the non-Hodgkin's lymphomas. A comprehensive staging study on approximately 200 patients jointly staged by the Medicine and Radiation Branches is nearing completion. In our series 74% of patients had Stage IV disease at completion of staging. Liver involvement and bone marrow involvement were the most frequent sites of visceral involvement.

Cell kinetics. Studies on the kinetic alterations on normal and malignant cells in animals and man have been performed.

A series of studies have been completed on the alterations induced by cyclophosphamide, BCNU, MeCCNU, cytosine arabinoside, cyclocytidine, 5-azacytidine, and adriamycin in normal and tumorous target tissues in vivo. All these agents show a differential sensitivity of the tumor compared to normal tissues and proper sequence of drug therapy can be designed around these kinetic differences. Both ascites tumor models (i.e., L1210, P388) and solid tumor systems (B16 melanoma) have been studied.

Studies on the effect of methotrexate on the suppression and recovery of DNA synthesis in human bone marrow and human ovarian carcinoma ascites cells have been completed in 6 patients with simultaneous study of blood and ascites concentration of methotrexate and clinical response. DNA synthesis in human tissues in vivo recovers when the concentration of methotrexate in the blood falls to 1 x 10^{-8}M or lower. Differential toxicity to normal and tumorous tissue can be used to predict clinical sensitivity or resistance to methotrexate in these patients with ovarian cancer.

Therapeutic trials. Forty patients have been randomized between dibromomannitol (DBM) and busulfan in order to investigate the comparative effectiveness of the former drug, a relatively new agent in a group of previously untreated Philadelphia chromosome positive recently diagnosed patients. The study has been completed and has required 5 years to properly evaluate this new agent in CGL. There were 16/20 complete remissions with DBM and 14/20 with busulfan. Failures were due to early blastic crisis and severe hypoplasia in 2 cases treated with busulfan. Mean time to remission was shorter with DBM (72 days) vs. 100 days for busulfan. Busulfan resulted in a more prolonged myelosuppressive effect such that 9/14 remitters required a median time of 9 months before drug could be readministered for maintenance compared with only 3/16 DBM patients who required a delay. Extensive histologic evaluation revealed no differences in the extent of cellular atypia produced in lung or bladder tissue. The survival for the groups did not significantly differ.

Treatment of blastic phase of chronic granulocytic leukemia. Since the demonstration that vincristine and prednisone were capable of inducing hematologic remission in 30% of a series of 30 consecutive patients with blastic crisis of CGL, a randomized trial of this combination vs. arabinosyl cytosine (Ara-C) (200 mg/M^2) and 6-thioguanine (200 mg/M^2) was begun. To date 32 patients have been studied. Thirteen have received vincristine, prednisone with 2 complete remitters in the latter groups have presented with hypodiploidy (44 Ph'). Including cases from our previous study 7/10 patients who presented with hypodiploidy at time of blastic crisis achieved a complete or partial hematologic remission. Crossover to opposite drug regimen was attempted in 22 cases with a partial response in 3 cases.

Laboratory investigations. The radionuclide ^{67}Ga has been extensively investigated. In summary, studies to date, some of which have been published, have revealed marked affinity of normal and leukemic granulocytes for the isotope. Further, this has been correlated with the presence of lysosomes. In addition, lymphocytes under the influence of PHA were shown to increase their capacity to bind ^{67}Ga which is a function of the appearance of lysosomes after PHA. Mouse melanoma cells have been studied by subcellular fractionation density gradients with marked localization of ^{67}Ga to melanosomes. Thus, we may have discovered a specific marker isotope for these organelles.

Antifolate pharmacology. This laboratory has been interested in the membrane transport of methotrexate in human neoplastic cells. Investigations in blast cells from approximately 15 patients with acute myelogenous leukemia, acute lymphocytic leukemia, and Burkitt's lymphoma suggest that methotrexate is transported into human neoplastic cells via an energy-dependent carrier mechanism at 10^{-6}M methotrexate concentrations. The carrier system appears saturable and has a K_m of about 6 x 10^{-6}M in ALL cells. Further, the observation that methotrexate and folinic acid compete for a common or closely-coupled carrier site for cellular entry has implications in the timing of "rescue" of methotrexate-poisoned cells. Additional studies have shown that the concomittant administration of other drugs to methotrexate-treated cells can inhibit or enhance the uptake of methotrexate by these cells. Hydrocortisone and Keflin both inhibit methotrexate uptake and vincristine enhances methotrexate uptake by retarding the exit of drug from the cell.

Methotrexate assay. Efforts of the current year have developed a competitive protein binding assay for methotrexate which is rapid (1 to 2 hours), sensitive (10^{-9}M) and highly specific for titrating inhibitors of dehydrofolate reductase. The assay is based on competitive binding of known, labelled ^{3}H-methotrexate and an unknown sample of methotrexate to bacterial reductase, with separation of bound and free drug by charcoal absorption. This assay also allows precise determination of the binding affinity of antifolates to reductase for the first time, and should be useful in quantitating this interaction for new folate analogs.

Studies with fluorinated pyrimidines. 5-Fluorouracil. Enzymatic assays for 5-FdUMP and dUMP have been developed and applied to the problem of determining the pharmacokinetic changes in these nucleotides associated with 5-FU administration in experimental animals and man. These changes have related the suppression and recovery of DNA synthesis in normal and malignant tissues in mice bearing P1534 leukemia and have revealed that the duration of inhibition produced by 5-FU is determined by the rate of disappearance of free FdUMP, as well as the rate of accumulation of the normal, competitive substrate, dUMP.

Medical Breast Cancer Service

An expanded multidisciplinary program in the study and treatment of breast cancer has been increased during the past year to include 7 medical beds in addition to the facilities and expertise of the staff of the Medicine Branch into which it has become incorporated. The program is involved in treatment programs at all phases of breast cancer and most are too early for analysis. However, in a prospective study substitution of adriamycin for methotrexate in the CMF program may be a more effective regimen for advanced disease.

The markers program, in collaboration with Dr. T. P. Waalkes of the Experimental Therapeutics Area, has indicated that 65% to 70% of patients pre- and postoperatively, and 97% of patients with metastatic disease, will have elevations of one or more of the following markers: human chorionic gonadotrophin, carcino embryonic antigen, or N^2, N^2-dimethylguanosine.

Hormones. Studies of steroid hormone receptors for all classes of steroid continue and are being correlated with clinical parameters and response to hormone therapy.

Studies of hormone dependence in vitro. Investigations have established and/or characterized numerous lines of human breast cancer, several of which exhibit marked dependence of various hormones including estrogens, androgens, insulin or growth hormone. Stimulation of macromolecular synthesis or cell division by physiologic hormone concentrations is demonstrable as well as the induction of specific breast marker protein alpha lactalbumin. Anti-estrogens strongly inhibit some of these cell lines. Hormone independent mutant cell lines are currently being prepared for analysis. These cells are also being grown on an in vitro capillary support system which will allow further characterization of hormone dependence.

Pediatric Oncology Branch

Educational. Pediatric Oncology Nurse. For the first time a 5-month course has been given to nurses in an attempt to develop a "Pediatric Oncology Nurse". This course was supervised in collaboration with the Nursing Service, CC, and involved the development of many new teaching techniques and concepts. On March 14, 1975 6 graduates of the program assumed their expanded role in the Clinical Center. It is our plan that this specialized education will lead to more effective patient care.

Leukemia antigens and the nature of the cell of origin of leukemia. ALL can be divided into at least two categories. The first group of patients, those whose cells do not stimulate in MLC, probably have a disease which represents proliferation of more mature T cells: those which had been under some thymic influence. The other group of patients probably have a disease which represents proliferation of the lymphoid stem cell in the bone marrow. It appears that the latter group are probably those with a better prognosis.

Therapeutic regimens in acute lymphatic leukemia. Previously untreated children with ALL were treated on a protocol designed to compare the effectiveness of maintenance chemotherapy alone vs. CT + immunotherapy. Patients received induction therapy with POMP followed by cranial irradiation with 2400R plus IT methotrexate or cytosine arabinoside. There was additional consolidation in half the patients with two 5 day courses of Ara-C and 6-thioguanine.

Maintenance randomization was either CT alone (4 months of intermittent prednisone plus vincristine, weekly methotrexate and daily 6-mercaptopurine alternating with 2 months of methotrexate alone) or to CT + IMT (4 months P, vincristine, methotrexate and 6 mercaptopurine alternating with 2 months if IMT) for 34 months. IMT was fresh Pasteur Institute BCG given by multiple puncture plus intradermal irradiated allogeneic leukemia cells from a single donor given in 4 weekly doses for the first month of the 2-month period. Forty nine patients were randomized for maintenance therapy. There was no significant difference in relapse rate (9/30 on CT and 7/19 on CT + IMT). Fifty nine percent of all patients are in complete remission at 30 months.

Blood component transfusion support (Clinical studies). Transfusion. During the past fiscal year 130 patients at the Clinical Center and 23 patients from San Francisco, Boston, Milwaukee, Baltimore and 5 greater Washington area hospitals were transfused with HL A selected four-unit platelet concentrates. To these 153 patients, 2,179 four-unit concentrates (8,716 units) were distributed: 1,905 to Clinical Center patients, 274 to non-NIH patients. The platelet procurement contract was awarded to Community Blood Services of Baltimore and the NIH Plateletpheresis Center was created on the reservation. The NIH Plateletpheresis Center during an 11 month period performed 2,769 procedures on 745 individual donors. The remainder of the plateletphereses were performed in the NCI Plasmapheresis Laboratory, as well as additional procurement studies using the Latham Blood Cell Processor.

Histocompatibility. The file of histocompatible (HL-A typed) donors has grown to 9,500 donors. Approximately 50 new individuals are HL A and RBC types each week. Blood was drawn from 2,840 prospective new donors for HL-A typing at the NIH Plateletpheresis Center during the contract period. Nine months ago, the National Cancer Institute implemented the "National Platelet Donor Pool", a computerized national blood component donor program.

Pre-clinical studies examining the utility of granulocyte transfusion for the treatment of Pseudomonas pneumonia in dogs treated with 350 rads of total body irradiation revealed that cells collected with the Cell Separator and by Filtration Leukapheresis were both effective in eradicating gram negative pneumonia. Equal numbers of long-term survivors were produced in both groups although twice as many granulocytes were transfused to recipients given cells collected by filtration leukapheresis. Studies of post-transfusion increments following infusion of filtration cells were lower on a per cell basis than those following transfusion of cell separator cells: correlating with similar observations in humans.

Bone marrow transplantation. Fourteen patients were transplanted with allogeneic or autologous bone marrow for the treatment of refractory leukemia, lymphoma or aplastic anemia. Four patients are alive 7+ to 56+ days. One patient remains in long term remission following transplantation with BACT (BCNU, CYT, Ara-C/60 g) chemotherapy for 3.5 years. A profound immunologic deficit described in last years' report has been completely corrected.

Studies on molecular mechanisms of productive infection and carcinogenesis associated with DNA and RNA tumor viruses. Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids have been developed.

Hamster cells transformed by nondefective Ad2-SV40 hybrids. Earlier efforts have isolated and characterized 5 nondefective Ad2-SV40 hybrid viruses ($Ad2^{+}ND_{1}$-$Ad2^{+}ND_{5}$) which contain different but overlapping segments of the

SV40 genome and small (2-5%) Ad2 deletions. Ad2+ND_1 induces SV40 U antigen; $Ad2^+ND_2$, U and TSTA; and $Ad2^+ND_4$, U, TSTA, and SV40 T-antigen. $Ad2^+$ ND_4 contains 43% of the SV40 genome, primarily the "early" region (associated with transformation). The SV40 segments have been mapped in relation to the wild-type SV40 genome by heteroduplex microscopy. Each of these hybrid viruses (as well as non-hybrid Ad2) transforms hamster kidney cells and such cells are oncogenic in newborn hamsters. The results indicate that interactions between the genomes of the nondefective hybrid viruses and the hamster cells they transform are random in some circumstances and non random in others. The findings may be applicable to recombinant DNA experiments in general, i.e., not all heterologous DNA introduced into eukaryotic or prokaryotic cells will be integrated; of that DNA which is integrated, some may not be transcribed, or if transcribed, may not be translated.

NCI-VA Medical Oncology Branch

Treatment of lung cancer has been continued by histologic cell types. In collaboration with the Working Party for Therapy of Lung Cancer it has been demonstrated that in limited epidermoid lung cancer, the Abrams-Cavanaugh short course radiation therapy gives results equal to more standard long-term course treatment.

For small cell carcinoma, the laminar air flow study has shown that high dose chemotherapy produces a higher response rate than does standard dose therapy; 95% vs. 60% respectively. The median survival also is increased in the high dose group. Infectious complications have been minimal. Combined radiotherapy and chemotherapy trials are also being conducted in collaboration with the WP-L. Results of these studies are still coded. Phase II trials with VP16-213 show a 30% response rate for advanced patients who have failed other chemotherapy regimens.

Laboratory studies. All new drugs in Phase I studies have their clinical pharmacology and pharmacokinetics evaluated simultaneously to provide maximal amounts of information regarding optimal dosing, drug schedules, and penetration into various sanctuaries of which the nervous central system is most important. Important preclinical information on a given drug is also obtained in the laboratory if it is already available. This year evaluations of VP16-213, thalicarpine, and isophosphamide on a daily x 3 schedule have been completed.

Preclinical studies. Preclinical studies in the solid L1210 system have been completed for the combination of phenformin (DBI) and BCNU. The former drug was chosen because it is mitochondrial poison that accumulates in acidic environments. Enhancement of BCNU effect was seen.

Project No. Z01-CM-03423-04-MO
1. Medical Oncology
2. Office of the Associate
   Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In Vitro Marrow Granulocyte Culture

Previous Serial Number: Same

Principal Investigator: Joan M. Bull, M.D.

Other Investigators: H. R. Pinedo, M.D., Annick Faille, M.D., Eleanor Stashick, Wilmarie McKoy, P. Carmeci, M. McMenamin, P. Schein, M.D., R. Kane, M.D., T. Chusid, M.D., S. Kassan, M.D., N. L. Gerber, M.D., J. Whang Peng, M.D., D. Zaharko, Ph.D., and D. Dale, M.D.

Cooperating Units: NCI-VA Medical Oncology Branch, MO,DCT,NCI, Medicine Branch, MO,DCT,NCI, Laboratory of Chemical Pharmacology, ET,DCT,NCI, Dept. of Technical Development, NIHLI, Clinical Immunology Sect., NIDR, Arthritis and Metabolism Branch, NIAMDD, Clinical Physiology Dept., and Laboratory of Clinical Investigation, NIAID.

Man Years:

Total: 4.5
Professional: 2
Other: 2.5

Project Description: (IXX)

Objectives:

1. Marrow and Circulating Peripheral Blood Granulocyte Cultures in Patients with Oat Cell Cancer of the Lung

The marrow and peripheral blood (PB) granulocyte proliferation (CFU-C) of patients with Oat Cell cancer are examined in vitro prior to tumor therapy to evaluate and compare with the marrow and PB CFU-C of patients with Squamous Cell carcinoma of the lung. These studies primarily attempt to distinguish between Oat Cell cancer patients with marrow invasion by tumor and those with tumor negative marrow by a difference in proliferative patterns of the marrow and PB CFU-C. In addition, these patients are being followed in a serial study with weekly PB CFU-C during therapy.

2. Chronic Neutropenia - Effect on Marrow and Peripheral CFU Kinetics

Marrow and PB granulocyte kinetics of patients with chronic neutropenia were compared with normal adults utilizing in vitro marrow proliferation combined with ^{3}H-TdR suicide techniques. The circulating PB granulocyte precursor was found to be in normal numbers in contrast to the decreased activity observed in neutropenic bone marrow CFU-C.

3. The Comparative Effect of Nitrosoureas on Marrow Proliferation

DCNU is an investigative nitrosourea with antitumor activity similar to BCNU and CCNU, but with minimal bone marrow toxicity in animal models. Comparative toxicities are tested on normal human marrows with both in vitro granulocyte culture studies and in vitro DNA and RNA synthesis.

4. Effect of Methotrexate Infusions on Marrow Proliferation

High dose methotrexate infusions to achieve constant high serum methotrexate dose levels are administered in vivo to mice. Marrow toxicity is evaluated by the murine in vitro marrow colony production. The purpose of these studies is to obtain toxicity information on high (10^{-5} to 10^{-8}) serum levels of methotrexate that have relevance to current clinical use of the drug.

5. The Effect of Synthetic Androgens on Granulocyte Proliferation

A study was done comparing the effect of synthetic androgens with a standard androgen, testosterone propionate, on marrow granulocyte proliferation. This study is ongoing to determine the cellular site of androgenic stimulation.

6. A Serum Granulocyte Inhibitory Factor in Patients with Felty's Syndrome

A controversy has existed whether the neutropenia observed in Felty's Syndrome is due to hypersplenism alone. It was observed that complement inactivated serum from rheumatoid arthritis patients with Felty's Syndrome inhibits autologous in vitro marrow proliferation whereas normal serum does not inhibit normal autologous marrow. This inhibitory factor appears to reside in the IgG component of serum.

7. Liquid Culture Studies

The Golde-Cline liquid culture technique is used to further examine effects on semi-solid marrow culture of cyclic AMP, GMP and hormonal influences of CFU-C proliferation.

8. CSA Assay

Ongoing assays continue to evaluate the active colony stimulating factor produced by Abbott Laboratories. It is intended that this serum-free factor will be produced in sufficient quantities to allow purification to enable antisera to be prepared for future studies.

9. Macrotube Scanner

In conjunction with the Technical Development Department, based on prior experience with a capillary tube system, we are developing a macrotube semisolid methylcellulose system that allows colony counting electrophotometrically. This system will allow a systematized direct early kinetic evaluation of marrow proliferation.

Future Course of the Projects:

The projects wil Felty's Syndrome, the macrotube scanner, studies with methotrexate and the nitrosoureas will continue. In addition, the androgens and other steroid modifiers of granulocyte proliferation will proceed by attempting to show nuclear binding sites for the hormones on the proliferatin colony forming cells and the CSF producing macrophages.

The effect of leukovorin rescue will be added to methotrexate studies in vitro and in vivo.

New work examining allogeneic lymphocyte stimulation of CFC-C is underway and will progress to the examination of this phenomenon in leukemia and lymphoma.

In addition, it is proposed to begin hyperthermic studies of marrow granulocyte proliferation and compare this to hyperthermic effects on solid tumor growth.

Publications:

1. Rosenblum, A. L., Bull, J. M., and Carbone, P. P.: Parameters of marrow proliferative capacity in vitro: Detection of a sex difference in normal human granulopoiesis. Blood 43: 841-846, 1974.

2. Waldmann, T. A., Bull, J. M., Bruce, R. M., Broder, S., Jost, M. C., Balestra, S. T., and Suer, M. F.: Serum immunoglobin E levels in patients with neoplastic disease. J. Immunol. 113: 379-386, 1974.

3. Rosenoff, S. H., Bull, J. M., and Young, R. C.: The effect of chemotherapy on the kinetics and proliferative capacity of normal and tumorous tissues in vivo. Blood 45: 107-118, 1975.

4. Bull, J.M., DeVita, V.T. and Carbone, P.P.: *In vitro* granulocyte production in patients with Hodgkin's disease and lymphocytic histiocytic, and mixed lymphomas. *Blood* (in press).

5. Mabry, J.R., Carbone, P.P. and Bull, J.M.: Amplification of colony stimulating activity in human serum by interaction with CSA from other sources. *Exper. Hematol.* (in press).

Keyword Descriptors: *In vitro* marrow granulocytes, colony growth, methylcellulose semisolid system, hematologic toxicity, colony stimulating activity (CSA), granulocyte/monocyte colonies, human serum in agar, conditioned medium, cytogenetics, androgenic steroids, non-adherent marrow, peripheral blood colony forming cell; hypaque sedimentation gradient, tritiated thymidine labeling index, autoradiograph technique, cyclic AMP.

Project No. Z01-CM-06119-07-MO
1. Cytogenetic Oncology
   Section
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cytogenetic and Hematopoietic Stem Cell Studies

Previous Serial Number: Same

Principal Investigator: Jacqueline Whang-Peng, M.D.

Other Investigators: George Canellos, Joan Bull, Paul Carbone, Douglass Tormey, Robert G. Graw, Jr., Ronald Barr, Richard Adamson, Susan Sieber, Harmar Brereton, Ralph Johnson, Harvey Gralnick, Henry Tan, John Neefe, Marvin Lutzner, Peter Wiernik, Joseph Fraumeni, Paul Gerber, P.M. Ming

Cooperating Units: Medical Oncology, DCT, NCI; Medicine Branch, MO,DCT,NCI; Pediatric Oncology Branch, MO,DCT,NCI; Laboratory of Chemical Pharmacology, ET, DCT, NCI; Radiation Oncology Branch, DCBD, NCI: Clinical Pathology Department, CC; Immunology Branch, DCBD, NCI: Dermatology Branch, DCBD, NCI; Clinical Branch, BCRC, DCT, NCI; Epidemiology Branch, DCCP, NCI; Viral Genetics Branch, BB, NIH; and Temple University, Phila., Pa.

Man Years:

Total: 4
Professional: 3
Other: 2

Project Description:

Objectives:

1. Cytogenetic studies (including 500-600 bone marrow and 300 peripheral blood specimens a year) in human neoplastic, hematological, and congenital diseases with special emphasis on: (a) leukemia patients who previously received large dose or total body irradiation for neoplasm; (b) patients with Sezary syndrome; and (c) patients with an initial diagnosis of myelofibrosis with myeloid metaplasia.

2. To observe the *in vivo* and *in vitro* effects of drugs and viral transformation. The major effort will be the study of chromosomal aberrations correlated with the teratogenic effects of Maytansine and viral transformation of human lymphoid cell line infected by EB virus and/or related viruses.

3. To study bone marrow grafting in the human, monkey, and dog by using different techniques.

4. To study the relationship of plasma cells, stem cells, and T and B cells in peripheral blood cells, and also study the growth and differentiation of stem cells to the myeloid, erythroid and megakaryocytic series.

Major Findings:

1. Bone marrow cytogenetic studies were performed on 30 patients (10 LSA; 10 Hodgkin's; 2 CLL, and 8 miscellaneous) who developed leukemia after receiving large doses of irradiation for previous malignancies. Up to 80% of these patients had aneuploid cells in all or some of their marrow cells. Four of these 30 patients had Ph' chromosomes. This high incidence of Ph' positive cases in irradiated patients is quite significant and is more than coincidental.

2. Of 31 cases of myelofibrosis with myeloid metaplasia, six developed leukemia of some kind; all of the latter had aneuploidy in their marrow cells. A seventh patient who died in an accelerated phase of his myelofibrosis also was aneuploid. Six months after diagnosis, the remaining 24 cases had not developed leukemia; 3 of these cases were in myeloproliferative disorder is obvious; the majority of the abnormalities involve the C group.

3. In a study of 17 cases of Sezary syndrome, there were 3 whose blood repeatedly failed to grow *in vitro*. Four out of the 14 successful cases showed definite aneuploidy in their lymphocytes, 5 had a few cells with aneuploidy, rearrangements, or structural aberrations. A total of 64% of the Sezary syndrome patients had chromosomal abnormalities.

4. Cytogenetic studies of Maytansine revealed a colchicine-like effect, with no gross chromosomal abnormalities or aberrations. *In vitro* lymphocyte test, showed that 50γ/cc of medium, caused complete mitotic inhibition while 10γ/cc had no effect on the mitotic index.

5. Stem cells from human peripheral blood can grow and differentiate into myeloid, erythroid, and megakaryocytic cells in diffusion chambers implanted in irradiated mice.

Publications:

1. Graw, R.G., Jr., Lohrmann, H.-P., Bull, M.I., Decter, J., Herzig, G.P., Bull, J.M., Leventhal, B.G., Yankee, R.A., Herzig, R.H., Krueger, G.R.F., Bleyer, W.A., Buja, M.L., McGinniss, M.H., Alter, H.J., Whang-Peng, J., Gralnick, H.R., Kirkpatrick, C.H. and Henderson, E.S.: Bone Marrow Transplantation Following Combination Chemotherapy Immunosuppression (B.A.C.T.) in Patients with Acute Leukemia. Transplantation Proc. 6: 349-354, 1974.

2. Sieber, S.M., Whang-Peng, J. and Adamson R.H.: Teratogenic and Cytogenetic Effects of Hycanthone in Mice and Rabbits. Teratology 10: 227-236, 1974.

3. Lohrmann, H.-P. and Whang-Peng, J.: Human Mixed Leukocyte Culture: Identification of the Proliferating Lymphocyte Subpopulation by Sex Chromosome Markers. J. Exptl. Med. 140: 54-60, 1974.

Keyword Descriptors: Cytogenetic studies, hemopoiesis, stem cells

Serial No. Z01-CM-06308-04 MO
1. Biostatistical Information
   Systems Unit
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1,1974 through June 30, 1975

Project Title: Biostatistical Information Systems Unit (BISU)

Previous Serial Number: Same

Principal Investigator: Richard Simon, Ph.D.

Other Investigators: None

Cooperating Units: Medicine Branch, MO,DCT,NCI; Pediatric Oncology Branch, MO,DCT,NCI; NCI-VA Medical Oncology Branch,MO,DCT,NCI; Immunology Branch, DCBD,NCI; Radiotherapy Branch,DCBD, NCI; Surgery Branch, DCBD,NCI; Division of Computer Research and Technology, NIH

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

The objective of the BISU is the utilization of statistical methodology and computer technology to maximize knowledge obtained from clinical trials and other research carried out within and on contract to the clinical programs of the Division of Cancer Treatment. The Unit participates directly in the design and analysis of clinical trials and other experiments, develops data collection procedures and related computer systems, and maintains computer files of collected data. The Unit also conducts research in statistical methodology in support of the above goals. A list of projects in which the Unit is actively involves follows.

1. Participation in the design, monitoring, and analysis of therapeutic clinical trials. The BISU monitors a contract for the abstraction and computerization of detailed patient data, and serves as the statistical center for design, randomization, data collection, and data analysis for all intramural therapeutic clinical trials in the Division of Cancer Treatment. The Unit also serves as the statistical center for collaborative clinical trials in ovarian cancer and metastatic breast cancer performed on contract to DCT.

2. Statistical collaboration in the design and analysis of retrospective clinical studies. In addition to numerous short consultations, collaborations have included: (a) development of a new clinical staging system for Burkitt's lymphoma; (b) evaluation of the clinical importance of early antigen and VCA in Burkitt's lymphoma; (c) evaluation of the prognostic importance of bone marrow involvement in advanced carcinoma of the breast; (d) evaluation of the prognostic importance of LDH level in patients with resectable Ewing's sarcoma; (e) evaluation of the prognostic importance of macrolymphoblasts in children with acute lymphatic leukemia; (f) evaluation of the relationship of quantitative changes in B or T lymphocyte populations to immunological responsiveness in patients with Burkitt's lymphoma; (g) evaluation of parameters predictive for the development of CNS leukemia in patients with acute lymphatic leukemia; (h) evaluation of the etiological and prognostic importance of the HL-A system in children with acute lymphatic leukemia; (i) evaluation of the immunological response to BCG in patients receiving chemotherapy for acute lymphatic leukemia; (j) evaluation of the effectiveness of CCNU in the treatment of Burkitt's lymphoma; (k) evaluation of the importance of proposed prognostic discriminants for patients with metastatic breast carcinoma; 1) evaluation of time sequence of protein production and DNA replication in lymphocytes; (m) evaluation of the prognostic importance of cardiac metastases in patients with non-resectable bronchogenic carcinoma.

3. The BISU serves as a general computer resource for the clinical programs of the DCT. A variety of computer files are maintained for the selection of platelet and granulocyte donors and for evaluation of the effectiveness of transfusions. A system is currently being developed for direct computerization, and analysis of scintillation counter paper-tape output.

4. Development and application of methodology for the thorough analysis of cell kinetic experiments in human and animal systems. We have been analyzing the effect of chemotherapy on the growth kinetics of rat hepatoma 3924A. We also serve as a consultant and assistant project officer of contracts for the study of cell kinetics in humans with breast cancer.

5. Biometric Research

a. Investigation of sequential termination and treatment assignment rules for the development of clinical trials which require fewer total patients and assign fewer patients the inferior treatment than is accomplished by traditional designs.

b. Development and evaluation of new methods of assigning treatments to patients in clinical trials with many stratification variables. Traditional methods may result in considerable imbalance of stratification variables for such situations. The methods developed here are now being utilized in many institutions.

c. Development of mathematical models for predicting effective ways of combining drugs or modalities. The prediction of these models have serious implications upon schedule design, and are currently being evaluated in the 13762 rat mammary tumors.

d. Development of statistical methods of adjusting for length-biased sampling in studies of disease etiology. These methods were developed for tissue antigen studies where the length-bias results because tissue typing is generally not performed at diagnosis.
e. Development of statistical methods for predicting which platelet donors who are almost HL-A matched to a patient will have family members who are exactly matched to the patient.
f. Development of biometric methods for improving the long-term transfusion support of thrombocytopenic patients by optimal assignment from pools of HL-A typed but not matched donors.
g. Development of Bayesian methods for the increasing of drug dosages in Phase I studies. These methods also have important applications to protected environment studies.

Publications:

1. Pocock, S. J. and Simon, R.: Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31: 103-116, 1975.

2. Simon, R., Weiss, G. H., and Hoel, D. G.: Sequential analysis of binomial clinical trials. Biometrika 62: (in press).

3. Simon, R.: Application of optimization methods to the hematological support of patients with disseminated malignancies. Mathematical Biosciences (in press).

4. Simon, R., Weiss, G. H., and Hoel, D. G.: A class sampling schemes for selecting the better of two binomial populations. J. Statistical Computation and Stimulation (in press).

5. Kovacs, C. J., Hopkins, H. A., Simon, R., and Looney, W. B.: Effects of 5-fluorouracil on the cell kinetics and growth parameters of hepatoma 3924A. Brit. J. Cancer (in press).

6. Brereton, H. D., Simon, R., and Pomeroy, T. C.: Pretreatment serum schemes for selecting the better of two binomial populations. J. Statistical Computation and Simulation (in press).

7. Leventhal, B., Levine, A., Graw, R., Simon, R., and Henderson, E.: Long-term second remissions in acute lymphatic leukemia. Cancer (in press).

8. Primack, A., Vogel, C. L., Kyalwazi, S. K., Ziegler, J. L., and Simon, R.: A staging system for hepatocellular carcinoma: Prognostic factors in Ugandan patients. Cancer (in press).

Keyword Descriptors: Statistics, data collection, computers, mathematical modeling.

Annual Report of the NCI-VA Medical Oncology Branch of the Division of Cancer Treatment, National Cancer Institute

Disease-Oriented Projects

Lung Cancer:

A multifaceted approach toward lung cancer was continued with progress made on several fronts. In diagnosis and prevention we have been attempting to better delineate high risk groups through studies of alpha 1 antitrypsin activity in sera, through studies of monocyte, lymphocyte aryl hydrocarbon hydroxylase activity, and also determination of HL-A types to determine unusual antigen frequencies in lung cancer. The alpha 1 antitrypsin study has been completed with negative results; the others are ongoing. The role of cytology in diagnosis was also extended by finding 100% concordance of cytologic diagnosis with histologic diagnosis for moderate and well differentiated tumors.

In staging of lung cancer we have evaluated the utility of ^{67} gallium citrate scanning. The role of this isotope was limited in that only large lesions were seen. Peritoneoscopy and bronchoscopy for staging were continued. We have also evaluated ACTA scans and ventilation-perfusion scanning for further accuracy in staging.

Treatment of lung cancer has been continued by histologic cell types. In collaboration with the Working Party for Therapy of Lung Cancer, we have demonstrated that in limited epidermoid lung cancer, the Abrams-Cavanaugh short course radiation therapy gives results equal to more standard long course treatment. We are currently employing fast neutron therapy in collaboration with MANTA. Such treatment may be beneficial because of the decreased oxygen dependency of high LET neutron therapy.

For small cell carcinoma, the laminar air flow study has shown that high dose chemotherapy produces a higher response rate than does standard dose therapy; 95% vs. 60% respectively. The median survival also is increased in the high dose group. Infectious complications have been minimal. Combined radiotherapy and chemotherapy trials are also being conducted in collaboration with the WP-L. Results of these studies are still coded. Phase II trials with VP16-213 show a 30% response rate for advanced patients who have failed other chemotherapy regimens.

For large cell anaplastic carcinoma and adenocarcinoma, patients with localized disease, are receiving combined modality therapy and patients with extensive disease are receiving only chemotherapy in prospective randomized trials in collaboration with WP-L. Results are coded. Results of preliminary Phase II trials with streptonigron and 6-thioguanine in these cell types have been negative.

Prostate Cancer:

Our present protocol shows responses of about 30% with CCNU and 5-FU. Equally important to therapeutic results have been attempts to define parameters of response in a disease where measurably masses are infrequent. Only serum and bone marrow acid phosphatase correlated with response or progression in all patients.

Hepatoma:

Activity of adriamycin in patients with hepatoma was evident. In addition, we have carefully evaluated biochemical parameters of response serially in these patients and have acquired more precise definitions of response.

Other Solid Tumors:

We are currently developing protocols for patients with squamous cell carcinoma of the head and neck and esophagus. Patients with a variety of solid tumors have been entered on ECOG protocols.

Phase I Trials:

Trials of thalicarpine and isophosphamide on a daily x 3 schedule every 3 weeks have been completed.

Disease-Related Projects

Evaluation of Response to Treatment:

Studies of biological markers have been carried out serially in patients with bronchogenic carcinoma. The two most promising markers are CEA and thyrocalcitonin. Marker levels seem to correlate with both response and progression of disease. The role of serial fiberoptic bronchoscopies and serial sputum cytologies in following tumor response continues to be evaluated. Morphometric studies are being evaluated for prognostic importance; morphometric index correlates with survival. Serial immunologic studies are being done to see if these predict response. In vitro bone marrow colony studies are being done in patients with small cell carcinoma to determine the effects of marrow involvement by small cell tumors on normal marrow colony forming units.

Supportive Care:

An intervention program for the nursing staff on our cancer ward is being carried out to enable the nursing staff to become more aware of their attitude toward death and dying so that closer and more open interpersonal contact among members of the nursing staff and the cancer patient may be fostered.

Nutrition:

Ongoing programs aimed at increasing caloric intake in cancer patients is ongoing and include: 1) taste testing with modification of high caloric supplemental formulas on the basis of taste tests, 2) hyperalimentation either orally or parenterally, 3) studies of nutritional patterns of cancer patients to determine patterns of dietary intervention that may benefit the patient.

Bacteriology:

Infection remains the major cause of death of the cancer patients. We have evaluated the use of countercurrent immunoelectrophoresis in rapid diagnosis of infection and find it to be an extremely valuable tool for identification of common microorganisms seen in cancer patients. The flora of the lower respiratory tract is a major source of infections. We have evaluated these organisms by fiberoptic bronchoscopy and translaryngeal aspiration with the latter procedure giving more reliable results. Fiberoptic bronchosocpy has also been shown not to predispose to bacteremia.

Laboratory Studies

Biochemical Pharmacology:

All new drugs in Phase I studies have their clinical pharmacology and pharmacokinetics evaluated simultaneously to provide maximal amounts of information regarding optimal dosing, drug schedules, and penetration into various sanctuaries of which the central nervous system is most important. Important preclinical information on a given drug is also obtained in the laboratory if it is not already available. This year evaluations of VP16-213, thalicarpine, and isophosphamide on a daily x 3 schedule have been completed.

Preclinical Studies:

Preclinical studies in the solid L1210 system have been completed for the combination of phenformin (DBI) and BCNU. The former drug was chosen because it is a mitochondrial poison that accumulates in acidic environments. Enhancement of BCNU effect was seen.

Cell Culture:

Both murine and human cell lines are being evaluated in cell culture. The human (Chago) line has been useful in that it produces several biochemical markers that can be followed in drug trials for determination of antitumor effect. New drug combinations that hopefully will be of clinical benefit are being developed in this system.

Project No. Z01 CM 03024-06 MO
1. NCI-VA Medical Oncology Branch
2. Washington, D.C.

PHS-NIH

Individual Project Report

July 1, 1974 through June 30, 1975

Project Title: Clinical Trials and Related Studies

Previous Serial Number: 3024

Prinicpal Investigators: Oleg S. Selawry
Martin H. Cohen

Other Investigators: Patrick J. Creaven, Mary J. Matthews, Lawrence E. Broder, Byron E. Fossieck, Robert C. Kane, Suryabala Kanhouwa, Marc J. Straus, Francisco Tejada, Robert Nelson, Augustin Schwarz, Barry Strauss, Paul Hamosh, and Bruno Gerstl

Cooperating Units: Medicine Branch, Biology Branch, Surgery Branch, NCI; Clinical Endocrinology Branch, NIAMD; Laboratory of Virus and Cancer Research, Department of Radiology, Cyclotron Branch, George Washington University School of Medicine; Pulmonary, Metabolic, Radiation Therapy, and Nuclear Medicine Sections and Surgery Service, WVAH.

Man Years:

Total:	74.25
Professional:	21.5
Other:	52.75

Project Description:

The overall aim of the NCI-VA Medical Oncology Branch is to improve the treatment and management of patients with malignant disease. While efforts are concentrated in patients with bronchogenic carcinoma, prostatic carcinoma and hepatocellular carcinoma, patients with all other types of malignancies are evaluated and treated by our staff. Efforts involve attempts at early diagnosis, accurate staging, definition of prognostic factors, supportive care, and combined modality therapy.

Key Words:

--- Lung cancer treatment
--- Prostate cancer treatment
--- Prostate cancer parameters of diagnosis
--- Hepatoma treatment
--- Phase I isophosphamide
--- Phase I thalicarpine
--- Death and dying: Intervention program for nursing staff

- Cell kinetics in solid tumors
- Biological markers in lung cancer
- Immunology in lung cancer
- Nutrition studies in lung cancer
- Pathology studies in lung cancer
- Scanning procedures in lung cancer
- Bronchoscopy studies in lung cancer
- Risk factors in lung cancer
- Bacteriological studies in lung cancer
- Morphometric studies in lung cancer
- *In vitro* bone marrow colony studies

Therapy Studies: Lung Cancer

Laminar Air Flow Protocol for Patients with Small Cell Carcinoma:

The protocol seeks to determine whether intensive therapy is more effective than standard therapy in the treatment of patients with small cell carcinoma and, further, whether the use of laminar air flow rooms and prophylactic oral non-absorbable antibiotics (PNAA) will protect patients from infection during drug induced leukopenia. Patients are randomized into the following groups:
I - high dose chemotherapy + PNAA + LAF
II - high dose chemotherapy + PNAA
III - standard dose chemotherapy

Thus far there is a total of 20 patients in groups I and II, combined since there has been no difference in response rates or infectious complications, and 10 patients in group III. The median survival for I and II has not been reached but would appear to be in the range of 8-11 months. For group III a median survival of about 5-6 months is likely.

Protocol for Patients with Limited Small Cell Carcinoma (WPL 72.21):

This protocol compares 2-drug chemotherapy (CCNU, cytoxan, and methotrexate) and chest radiation to 5000 Rads, with the same chemotherapy and chest radiation plus brain irradiation to 3000 Rads. Response rates are coded at present, but are 74% on one arm and 58% in the other. Corresponding median survivals are 31 weeks and 23 weeks, respectively. It is too early to evaluate the role of brain radiation in preventing CNS metastases.

Protocol for Patients with Extensive Small Cell Carcinoma (WPL 72.22):

This protocol compares 3-drug chemotherapy (CCNU, cytoxan, and methotrexate) with the same chemotherapy plus radiation of brain (3000 Rads), chest (2000 Rads), and abdominal (2000 Rads). Responses are coded, but are 26% in arm and 50% in the other. Median survivals are 20.5 weeks and 18.6 weeks respectively. Greater patient accrual is needed before final conclusions can be made.

Protocol for Patients with Limited Epidermoid Lung Cancer: Standard vs. Short Course Radiotherapy (WPL 73.12):

This protocol compares "standard" radiotherapy of 3000 Rads over 3 weeks and an additional 2000 Rads over 2 weeks after a 1 to 2 week rest with a short course of radiotherapy of 2000 Rads in 5 days followed by 3 weeks rest followed by 2000 Rads over 2 weeks. One hundred and thirty-four patients have been entered. Thus far response rates and median survivals of both treatment regimens are equal. If these results continue and are accompanied by objective evidence of local tumor control, the short course will represent a means of delivering treatment involving less time and expense.

Therapy of Limited Extent Adenocarcinoma and Large Cell Carcinoma of the Lung (WPL 73.31):

The purpose of this protocol is to compare the efficacy of radiotherapy alone with radiotherapy plus chemotherapy (cyclophosphamide, methotrexate, CCNU) in the treatment of locally advanced but regional adenocarcinoma and large cell carcinoma of the lung. As of March, 1975, 19 patients have been entered on the protocol from the NCI-VA Oncology Unit and 29 patients have been entered overall. The protocol results remain coded. Ambulatory patients survive significantly longer than non-ambulatory patients. Results of the two treatments are not significantly different in survival time, although survival for responders is significantly better than that for non-responders. Pronounced or life-threatening toxicity was seen in 16/28 (57%) patients on the radiotherapy plus chemotherapy arm while only 4/19 (21%) exhibited this toxicity on the radiotherapy alone arm of the protocol. The study remains open to patient accrual since only 40% of the estimated 200 patients needed to complete the protocol have been entered.

Extensive Large Cell and Adenocarcinoma (WPL 74.52):

This study compares two virtually non cross-resistant regimens, CCNU, cytoxan, methotrexate; and adriamycin and procarbazine, with a cross-over of progression. The treatment regimens are still coded. Regimen I has an overall 18% response rate with a median survival of 13.8 weeks, regimen II a 30% response rate with 21.9 weeks median survival. Toxicity is somewhat more severe for the adriamycin-procarbazine combination.

Neutron Radiotherapy (MANTA):

The oxygen effect is a fundamental characteristic of the radiation response of mammalian cells. Certain solid cancers of laboratory animals are known to contain hypoxic and relatively radioresistant cells which are clonogenic. It is believed human solid cancers have similar characteristics. Human cancer radiotherapy results with low LET cobalt or x-ray radiations provide human radiobiological evidence that, in respect to the radiation response the important clonogenic cells of certain human solid cancers are radioresistant. Such radioresistance could be attributed to the oxygen effect. If these considerations do reflect the human situation, then neutron radiotherapy (relatively high LET and less oxygen-dependent radiotherapy) may be accompanied by an improved radiation response and a greater local control. Consequently, fast neutron radiation therapy studies are ready to be initiated in patients with epidermoid cancers of the lung and head and neck.

Phase II Protocol for Patients with Lung Cancer:

Epidermoid carcinoma (Emetine hydrochloride): The phase I, phase II, emetine study is nearing completion. The moderately toxic dose was 100 mg/m^2 IV q week. Twelve patients have been entered at this dose with no responses.

Large cell anaplastic (Streptonigran, 1 gm/m^2 PO biweekly): There were supply problems with this drug and hence data is too preliminary for reporting.

Small cell carcinoma (VP16-213, 200 mg. IV q weekly): Ten patients have been entered, with 3 responses of over 2 months duration. This looks like a promising finding with regard to a new drug for small cell carcinoma.

Adenocarcinoma (6 thioguanine, 75 mg/m^2 PO q daily): No responses have been seen in 7 evaluable patients. There has been no unexpected toxicity.

Chemotherapeutic Trials in Patients with Cancer of the Prostate:

A controlled prospective study was initiated in September, 1973, to evaluate the response to chemotherapy of patients with prostatic cancer in two groups: Group I, untreated Stage D patients; Group II, patients with previous hormonal manipulation.

Twenty-one patients have been entered for a total of 25 trials, as follows:

Group	Treatment	No. Entered	No. Evaluable	C.R.	P.R.	Progression
I	DES	5	4	0	4	0
	DES + 5-FU	3	2	0	2	0
II	CCNU	9	8	0	3	5
	5-FU	8	7	0	2	5

Thus all treatments appear active. Precise evaluation of the role of chemotherapy will require survival data that we are currently accumulating.

Hepatoma (Phase II Trial):

The purpose of the study is to determine the response rate of hepatomas to adriamycin. Pretreatment, factors possibly influencing response (presence or absence of cirrhosis, HB_5Ag, Alpha feto protein, hemochromatosis, chronic alcoholism) are determined, and multiple parameters of response (liver scan, tumor markers, physical examination, liver enzymes-LDH, isoenzymes, SGOT, alkaline phosphatase, 5' nucleotidase, malic dehydrogenase, phosphohexose isomerase, aldolase, and beta glucuronidase) are being obtained serially to identify useful parameters of response. To date, 7 patients have completed the first course of adriamycin, 60 mgm/m^2 IV q 3 weeks, 3 responses have been observed.

Initial Clinical Trial of Isophosphamide:

A phase I clinical trial of a daily x 3 dose administration of isophosphamide, repeated once at an interval of 21 days, was started at an initial daily dose of 1600 mg/m^2 body surface area, given as an IV infusion in normal saline over 30 minutes. Each patient was assessed for adequate hepatic and renal function pretreatment, and was started on IV hydration at a rate of 100 ml/hr/m^2 at least 12 hours prior to the first dose to ensure adequate urine flow; hydration was continued 24-36 h. after the last dose. The purpose of the 3-day divided dose regimen was to attempt to avert the dose limiting toxicity from hematuria seen with high single doses. Bladder irrigation was not employed in any patient in this study. Eight patients were treated at the initial dose range of 1600 mg/m^2, and 12 patients were later entered at an escalated range of 2400 mg/m^2 employing the same schedule as with the lower dose range.

Hematologic toxicity was seen in only one-half of patients in both dose ranges, and was very mild (nadir at 11 days, WBC mean nadir 2900). One patient who had extensive prior chemotherapy and treated at the high dose range developed prolonged thrombocytopenia (platelets 45,000 for 12 days). Alopecia occurred in 75% of patients, and again was not related to dose range. Nausea and vomiting occurred in 5/8 patients at the low dose and 4/12 patients at the high dose. One patient treated at the high dose had a subarachnoid hemorrhage on day 4 of therapy and subsequently developed acute tubular necrosis, oliguric renal failure, and shock, and expired; drug toxicity may have contributed to the cause of death. CNS toxicity, manifested by mild somnolence and/or excitement was observed in 25-50% of patients and was readily reversible.

One of 8 patients treated at the low dose range had unacceptable dose limiting toxicity (psychotic reaction in a patient with past history of psychosis); five of 12 patients had unacceptable toxicity at the high dose range (1 prolonged thrombocytopenia, 1 acute tubular necrosis, 3 gross hematuria). For this daily x 3 divided dose regimen, a daily dose of 2400 mg/m^2 appears to be the maximally tolerated dose.

Phase I Studies; Phase I Study of Thalicarpine:

The Phase I study of thalicarpine has been completed. This compound, aprophine benzylisoquinoline plant alkaloid of novel structure was entered into initial clinical trial because of its activity against the Walker 256 carcinosarcoma, and because in preclinical testing it had proved to have no myelosuppressive toxicity. Such drugs are always of great potential interest because of their possible use in combination. A total of 36 patients were studied. Thirty-three single IV doses were given to a total of 28 patients , and a total of 14 weekly courses were given. The single IV doses ranged from 200 mg/m^2-1900 mg/m^2 of body surface area. It was considered that 1400 mg/m^2 as single dose was the clinically maximally tolerated dose. At this dose, the toxicity consisted of arm pain in 1 of 10 patients, on occasion severe enough to necessitate restarting the infusion in the other arm; CNS depression in 7 of 10 patients, usually drowsiness but in 1 patient progressed to semicoma. Nausea and vomiting was milk occurring in only 2 patients and the principal other toxicity were subtle EKG changes mainly T wave flattening which recovered within hours after stopping the infusion. The maximally tolerated dose for weekly administration was considered to be 1100 mg/m^2/week for 6 weeks. At this dose the same types of toxicity was seen but were milder. There was no evidence of cumulative toxicity over the 6-week period. There was no hepatic or renal toxicity seen and no evidence of myelosuppression. For these reasons, we feel that thalicarpine is a drug worthy of further evaluation because of its potentiality for use in combination chemotherapy. A dose of 1100 mg/m^2/week by 2-hour IV infusion is recommended for further studies.

Participation in Cooperative Studies, Acute Leukemia Group B (ALGB):

A total of 11 patients were entered on the following ALGB protocols: prevention of blast crisis in CML (1 patient); treatment of advanced lymphosarcoma and reticulum cell sarcoma (1 patient); primary treatment of multiple myeloma (2 patients); induction therapy of untreated AML (4 patients); and primary treatment of multiple myeloma (3 patients, No. 7461).

Chemotherapy of Extensive Epidermoid Carcinoma of the Lung:

A study protocol utilizing a triple combination of methotrexate, bleomycin, and streptozotocin for the treatment of extensive epidermoid carcinoma of the lung was proposed and initiated in May, 1975. The rationale is the use of agents that have independent and non-additive toxicities to maximize therapeutic index. The agents will be given to good risk patients (performance status of 2 or better on the Zubrod scale) and will be limited to patients less than 70 years of age. Continuous therapy using weekly streptozotocin and biweekly doses of bleomycin and methotrexate will be employed. The patients will be treated for a minimum of 6 weeks for estimation of therapeutic effect as well as toxicity. Parameters of effect will include objective response rate, time to response, duration of response, and survival. It is estimated that at least 25 patients will be needed for analysis of effect if there are responses seen in the first nine patients. Further evaluation of effect via randomized studies will be undertaken once therapeutic effect without untoward toxicity is established.

Eastern Cooperative Oncology Group:

Seven patients have been entered into protocols of the ECOG: head and neck (4 patients); weekly methotrexate, vs. methotrexate and leukovorin, vs. methotrexate, leukovorin, cytoxan and ara C. Sarcoma study (3 patients); adriamycin vs. adriamycin, vincristine, cytoxan, vs. vincristine, cytoxan and actinomycin D.

Patient Census Data:

Admissions:	447
Discharges:	432
ADPC:	24.3
Out-Patients:	142.3 (average)
Clinic Visits:	2251
Consults:	325
Deaths:	94
Autopsies:	69
Patients Entered on Study:	105

Patients by Disease Type:

Leukemia:	8
Lymphoma:	19
Lung:	135
Other Solid Tumors:	90
Myeloma:	8
Central Nervous System	4
Breast Cancer:	7
Miscellaneous	110

Effects of an Intervention Program for Nursing Staff on a Cancer Ward:

The general purpose of the investigation is to assess the effects of a direct approach toward open communication and desentization in the area of death and dying, with the goals of increased, closer, and more open interpersonal contact among members of the nursing staff and between the nursing staff and the terminal cancer patients on the ward. The method used was a planned series of discussions in small groups with open-ended questions and role-playing as techniques at the discretion of the leader, to stimulate expression of attitudes and feelings about death and dying. All 32 members of the nursing staff of the NCI-VA Medical Oncology Section will participate in one-hour weekly sessions led by a clinical psychologist. The content of the sessions will be descriptively summarized by the leader. Evaluation will be made of changes in attitude and behavior by questionnaire, essay questions, and observations before and after the series of group sessions. If the data indicate positive results, a continuation of the sessions as a part of the Oncology program will be recommended.

Solid Tumor Cell Kinetics:

A total of 13 patients have been studied. A patient with cancer of the penis was studied with evaluation of dosimetry and monitoring of systemic ^{3}HTdR. Seven patients with lung cancer and 5 patients with head and neck cancer have been studied with evaluation of dosimetry of systemic ^{3}HTdR and cell kinetics of primary and metastatic tumor.

The studies show that 33% of the injected dose of ^{3}HTdR radioactivity was excreted in urine in 24 h. and 55% total in 10 days. The plasma activity decreased to 20% of initial levels in 10 minutes. Plasma activity decay was then constant. Urine activity peaked in 6 h. at 10-20 times peak plasma activity. Urine levels equaled plasma's at 1-2 days. Activity of air vapor and sputum increased to plasma levels in 1 h. The regression of plasma, air, and sputum activity after 1 h. and urine after 1-2 days measured over 2 months was linear; T 1/2=10-12 days. Autoradiographs for cell kinetic data are being evaluated and will be used for planning of future therapeutic trials.

Calcitonin Production in Patients with Bronchogenic Carcinoma:

Following the initial positive results of a pilot study begun in 1973, a prospective study was initiated in July 1974. As of December 1974 a total of 35 patients have been evaluated with 18 collected through March 1975 which are unevaluable at this time. Of the 35 evaluable patients, there were 10 epidermoid, 11 small cell, 10 adeno., and 4 large cell carcinoma patients in the series. Twenty-six patients had extensive disease while 9 patients had limited disease. Elevated values for calcitonin (above 300 pcg/ml) were observed in about 75% of patients in each group irrespective of cell type. Several patients have been catheterized for venous effluent blood from both the tumor and thyroid gland. This data is still being evaluated. With 53 patients entered on study, further accrual has been stopped.

Biologic Markers in the Evaluation of Disease Status of Patients with Advanced Bronchogenic Carcinoma:

A series of 69 previously untreated patients with regional or extensive unresectable bronchogenic carcinoma were screened and followed serially at two week intervals with one or more of the following markers: Alpha-feto protein, CEA, and HCG. No increase in alpha-feto protein was noted. CEA was increased (above 5 ng/ml) in 45% and 66% of regional and extensive bronchogenic carcinoma respectively while HCG was present in 10.5% of each respectively. There was no correlation between CEA or HCG level and number of metastatic sites or symptom duration although high CEA values (above 50 ng/ml) were associated with shorter survival durations in non-responders. Eight of 9 responders with initial CEA elevations demonstrated an average 41.6% decrease at maximum response while 1 of 3 with normal initial levels decreased at response. Nine of 15 non-responders or subsequent progressors with initial CEA elevations demonstrated an average 64.9% increase at first clinical progression. Thus CEA may be useful in evaluating response and progression in these patients.

Immunology of Cancer:

Through cooperation with the Immunology Branch, various parameters of immune response in 2CN protocol patients will be assessed. These include MIF, and blastogenic response to mitogens and allogeneic cells. A non-specific immune adjuvant trial utilizing C porvum is planned. Dose scheduling and route and their effects on immune parameters by C parvum is currently under way in a dog model. An assay for macrophage killer function is likewise being developed as an additional parameter to assess the effect of C parvum. It is anticipated that results of these studies may influence future protocol designs.

Nutrition Studied in Cancer:

Taste tests: Four primary modalities (sweet, sour, bitter, and salt) are tested. Cancer patients have lesser sensitivity to sweet, salt, and sour with normal sensitivity to bitter.

Nutritional intakes: Dietary intake has been plotted for 100 patients to include total calories, proportions of calories as fats, carbohydrates and proteins, amounts of vitamins and trace elements ingested and proportion of total days calories taken at each meal. Overall caloric intakes were sufficient for a non-ambulatory patient but weight loss continued. Nitrogen balance studies are planned.

Nutritional supplementation: Based on the results of the above taste tests we are attempting to match the taste of various nutritional supplements to taste patterns of the patient with the aim of increasing weight.

Effect of non-absorbable antibiotics on dietary intake on the laminar air flow project: Patients on antibiotics decrease their caloric intake but do not appear to have significant problems with drug induced malabsorption.

Cytologic Subtyping of Lung Cancer:

Prior studies of positive lung cancer cytology, classified into four major cell types, demonstrated that there was a 100% accuracy consisting between tissue and cytologic diagnosis of moderately and well differentiated tumors, and little or no accuracy in identification of poorly differentiated tumors. In the past year, positive cytology has been classified by subtype according to the WPL classification in an attempt to improve accuracy of poorly differentiated tumors. These, studied to date, suggest that cytology subtyping is not only consistent with but frequently more accurate than tissue diagnosis, particularly when the lattter are based on small crushed biopsies obtained by fiberoptic bronchoscopy.

Cardiac Metastases in Lung Cancer:

An overall rate of 25% of 418 patients with lung cancer was proven to have pericardial and/or myocardial metastases at autopsy. This phenomonon was particularly noted in patients having poorly differentiated tumors and extensive disease. Thirty-eight of 114 patients on aggressive NCI-VA chemotherapy-radiotherapy protocols had cardiac involvement. Forty-one percent of these patients with adenocarcinoma and 59% of patients with large cell carcinomas had cardiac metastases. Five of the 38 patients were suspected to have cardiac disease because of increasing congestive failure or sudden enlargement of the cardiac shadow. Two of these five were diagnosed prior to death, were treated appropriately, and discharged to home.

Retrospective Clinics-Pathologic Study of Prostatic Carcinoma:

A retrospective study of over 200 patients with carcinoma of the prostate, identified or confirmed at autopsy is in progress. Information assembled to date includes the fact that the disease had been recognized and variably treated in approximately one-third of the cases. The tumor had locally eroded or disseminated sufficiently in another 15% of the cases, so that clinical diagnosis should or might have been entertained in approximately one-third of the cases had another primary malignancy (predominantly the lungs and gastrointestinal tract). Evaluation of grading and histologic patterns of primary and metastatic tumors and the correlation of osteoblastic and osteolytic bony metastases with serum alkaline and acid phosphatase levels are in progress.

Reliability of Histologic Diagnosis:

In collaboration with two other pathologists, material has been reviewed from 165 patients of the Working Party for the Therapy of Lung Cancer and 200 patients from the VA Surgical Adjuvant Group. Additionally, material from a joint ECOG-RTOG small cell study will be reviewed. Overall accuracy for epidermoid carcinoma was 85% and for adenocarcinoma and small cell carcinoma, 95%.

Scanning Procedures:

ACTA scanning procedure: We are currently evaluating the role of the ACTA scanner (Automated Computerized Transverse Axial) tomographic scanner in evaluating disease of the chest and also in evaluation of clinically non-apparent disease in the abdomen. It is too early to determine the value of this procedure.

Ventilation/perfusion scanning procedure: To aid the evaluation of chemotherapy, accurate quantitative measurements are needed. Recently employed methods in evaluating lung tumor size are subjective, and consist of visual assessment of tumor size using the x-ray of the chest. In this study, ventilation/perfusion dynamic scan using Xenon^{133} before and after chemotherapy in a selected group of patients, is compared to x-ray evaluation of tumor. We adapted the apparatus, which consists of an Anger camera interfaced with a PDP 11/40 computer, to our needs. Some of the software is still being developed. Preliminary studies in patients are presently in progress. Evaluation of data is premature at this time.

Bronchoscopy:

One hundred and fourteen bronchoscopies have been done this year in order to: 1) obtain histologic proof of malignancy, 2) obtain serial biopsies for cell kinetic studies, 3) evaluate the microbial flora of the lower respiratory tract 4) have a parameter of response to serially follow tumor size, 5) obtain secretions for marker studies, and 6) to obtain alveolar marcophages for biochemical studies. All of these studies are progressing in a satisfactory manner.

In Vitro Colony Forming Activity of Bone Marrow from Patients with Small Cell Carcinoma of the Lung:

Bone marrow involvement by tumor is present in about 45% of patients with small cell carcinoma of the lung at the time of diagnosis. In vitro marrow granulocytic colony formation is being quantitated serially in patients before and during therapy in patients with and without marrow metastases diagnosed histologically. Correlations are being investigated between presence or absence of histologic evidence of marrow metastases, pretreatment, tolerance to chemotherapy, and subsequent resolution occurrence of bone marrow metastases. To date, 4 patients with limited epidermoid carcinoma of lung (with involvement of marrow) have had pretreatment marrows cultured as control, and 12 patients with small cell carcinoma have had pretreatment marrow cultures. Four patients have been followed with repeat marrow cultures at six weeks of therapy. In patients with metastases, colony forming activity was depressed.

Risk Factors for Lung Cancer:

Alpha-1-antitrypsin: Alpha-1-antitrypsin deficiency in both homozygous and heterozygous forms has been associated with an increased incidence of chronic obstructive pulmonary disease. To see whether deficiency of this enzyme was associated with an increased incidence of lung cancer, 73 patients were studied. All had normal or high enzyme levels and, therefore, alpha-1-antitrypsin deficiency is not likely associated with an increased cancer incidence.

Aryl hydrocarbon hydroxylase (AHH): This inducible enzyme has been shown to be increased in lymphocytes and monocytes of lung cancer patients and in alveolar macrophages of smokers. The role of this enzyme in respiratory carcinogenesis or anti-carcinogenesis remains to be more fully evaluated. We are currently measuring monocyte levels and hope to extend these studies to bronchial mucosa.

Vitamin A: Vitamin A deficiency is associated with squamous metaplasia of respiratory epithelium and with increased binding of respiratory carcinogens. We have evaluated serum vitamin A levels which are normal in our patients. Mucosal values remain to be determined.

HLA typing: To determine if any HLA types are at increased risk of lung cancer we are evaluating our newly diagnosed patients. Ninety-five such patients have been entered thus far. It is too early for results.

Morphometric Studies of Pulmonary Cancer:

Subsequently, prognostication of pulmonary carcinoma has been attempted by considering the various cellular types, vascular invasion, mitotic index, growth rate and performance status. The latter shows good correlation with average survival time of groups of patients, but does not allow prognostication of individual cases. Quantitative microscopy is used for this purpose. Morphometry was supplemented by the mitosis:necrosis ratio, reciprocal of cell count and number of apoptosis bodies. Data obtained on 15 short term (4-19 mo) and 8 long term (30-132 mo) survivors with epidermoid carcinoma were expressed as indices. The morphometric index averaged 106.8 for short term and 156.1 for long term survivors respectively. Addition of the other three indices resulted in an average of 171.4 for short and 318.5 for long term survivors.

Bacteriology Studies:

Usefulness of countercurrent immunoelectrophoresis in rapid diagnosis of infection in patients with cancer: This procedure detects bacteria antigens in body fluide (CSF, serum, urine, pleural fluid, etc.) down to concentration of about 20 ng/ml within 30 minutes. Established effective antisera are available K. pneumoniae, S. pneumoniae, H. influenzae, N. meningiditis. Antisera without established effectiveness or in the developmental stage are P. aeroginosa, S. marcesens, C. albicans. Established effective antigens for the detection of antibody are S. aureus techoic acid. To date the following number of specimens have been examined:

	No. Done	No. Positive
S. pneumoniae	50	29
K. pneumoniae	7	2
S. aureus	10	2
C. albicans	3	0
N. meningiditis	4	0
H. influenzae	0	0
Ps. aeroginosa	5	1

A comparison of the local and infectious complications of fiberoptic bronchoscopy and translaryngeal aspiration: Eighty-one fiberoptic bronchoscopies were evaluated for fever, bacteremia, hemorrhage, etc. Fever, 5; bacteremia, 1 in patient with pneumonitis; hemorrhage, 2. Fifty-four translaryngeal aspirations were evaluated for subcutaneous emphysema, fever, hemorrhage, local infection, etc. Fever, 0; subcutaneous emphysema, mild, 33; marked, 1; hemorrhage, 1 (platelet count, 10,000); and local infection, 0. The study indicates that bacteremia does not occur following fiberoptic bronchoscopy unless active infection is present. Procedures are safe and well tolerated and relative risk should be weighed against value of results.

Lower respiratory tract study: The study was designed to evaluate 1) lower respiratory tract microflora of lung cancer patients and their possible role in infection and 2) accuracy of fiberoptic bronchoscopy and translaryngeal aspiration in obtaining culture specimens. FY 1975, 23 studies on 14 patients. Overall to date: 10 of 42 translaryngeal aspirations showed significant colonization, and 32 of 42 translaryngeal aspirations were sterile or had insignificant growth; and of the fiberoptic bronchoscopy specimens obtained immediately following translaryngeal aspirations, 29 of 42 fiberoptic bronchoscopies showed significant colonization and 12 of 42 fiberoptic bronchoscopies were sterile or had insignificant growth.

Many more kinds of bacteria, especially potentially pathogenic aerobic and anaerobic gram negative rods were grown from fiberoptic bronchoscopy specimens and tended to reflect results of throat cultures. When infections occurred, they were caused by microorganisms initially present in the translaryngeal aspiration specimens. Fiberoptic bronchoscopy culture results could be misleading and inaccurate.

Streptococcemia in cancer patients: The study was initiated to learn 1) the frequency of Gram positive coccemia in patients with cancer, 2) the predisposing factors that lead to suspicion and occurrence of Gram positive coccal infections, 3) efficacy of various diagnostic procedures, and 4) effect of therapy. Data to date obtained in 15 of 20 streptococcemias show: 12/15 solid tumors and microorganisms; 7/15 S.pneumo.; 2/15 Group A strep.; 1/15 Group B strep; 1/15 Group D strep.; 3/15 anaerobic strep; and 1/15 strep viridans.

Parameters of Response in Chemotherapy Treatment of Prostate Cancer:

While treating stage D adenocarcinoma of the prostate with chemotherapy agents we are attempting to find what parameters are indicative of tumor response or progression. The parameters being evaluated are: measurable tumor, performance status, weight, hematocrit, peripheral blood alkaline and acid phosphatase, bone marrow acid phosphatase, bone survey and scan. The results of 16 evaluable trials to date are: 4 had measurable tumors with 2 objective tumor regressions and 2 progressions. Hematocrit and peripheral blood alkaline phosphatase were of no value. Changes in the other parameters were correlated with response or progression of disease as judged by overall clinical state of the patients. There were 7 responses, 8 progressions, and 1 stabilization of disease. In the parameters that returned toward normal only the peripheral blood and bone marrow acid phosphatase correlated with disease response. In the parameters that deviated further from normal, only the bone marrow acid phosphatase correlated with disease progression. While performance status, weight, peripheral blood acid phosphatase, bone survey and scan were useful, they did not always correlate with the pattern of response of an individual patient. Only measurable tumor and bone marrow acid phosphatase changes were consistent, in all cases with response or progression of disease.

Education, Training, and Dissemination of Information

Scientific and educational efforts of the staff are reflected in 33 abstracts and over 200 intramural and extramural lectures on seminars, including the following:

--- two weekly one-hour conferences dealing with basic science and/or clinical aspects of oncology;
--- two weekly one-hour lectures on oncology to nursing staff by a second-year clinical associate;
--- twelve presentations at the Medical Oncology weekly conferences at NCI;
--- twelve presentations at the Washington VA Hospital Medical Service conferences;
--- six lectures at GW University School of Medicine.

A total of 30 George Washington School of Medicine students participated in 5 Physical Diagnosis courses led by 5 oncology staff members. Dr. Byron Fossieck Oncology senior staff member, supervised all Physical Diagnosis courses at the VA Hospital.

A total of sixteen Oncology and Pathology residents and medical students participated in a total of 36 seminars on pathology and cytology given by the Oncology Senior Pathologist.

Seven seminars for a total of seven dietitian specialists and twelve students were led by the Oncology Dietitian.

A total of 30 nursing students from George Washington University School of Nursing and Catholic University School of Nursing participated in Oncology ward activities as part of the training, co-supervised by the Oncology Head Nurse.

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Project No. Z01 CM 06309-06 MO
1. NCI-VA Medical Oncology Branch
2. Washington, D.C.

PHS-NIH

Individual Project Report

July 1, 1974 through June 30, 1975

Project Title: Laboratory Studies in Cancer

Previous Serial Number: Formerly part of 3024

Principal Investigator: Patrick J. Creaven

Other Investigators: Larry M. Allen, Martin H. Cohen, Lawrence E. Broder, Robert Nelson

Cooperating Units: Laboratory of Theoretical Biology, DCBD; Clinical Endocrinology Branch, NIAMD; Metabolic Section, WVAH

Man Years:

Total:	6.5
Professional:	3.5
Other:	4

Project Description:

Laboratory studies in biochemical pharmacology, cell cultures, and preclinical projects have been planned in conjunction with the clinical studies (serial number 3024), thereby ensuring an integrated research program in the Medical Oncology Branch.

The Oncologic Pharmacology Unit conducts investigations primarily of new anticancer agents but also carries out some studies of established agents. It is responsible for the Phase I testing of new agents in the Branch and for conducting clinical pharmacology and biochemical pharmacology studies. In addition, because we feel that the value of clinical pharmacology studies is often lost through inadequate interpretation of the data generated, we have devoted a considerable effort to developing pharmacokinetic modelling of anticancer drugs using the extensive computer facilities of NIH. The work started with single dose ifosfamide has been continued with daily x 3 ifosfamide without, however, altering the nature of the dose limiting toxicity as had been hoped. Studies on thalicarpine were completed; an MTD for single dose and weekly administration has been reported and pharmacology studies have shown a long body retention of the drug. A major effort has been made in the investigation of the pharmacology and pharmacokinetics of the epipodophyllotoxins VP16-213 and VM26. Interesting pharmacokinetic differences between the two drugs have been found and pharmacokinetic models are under development.

The cell culture laboratory using a cell line derived from a lung cancer patient has been involved in the determination of the effects on chemotherapy, hormone production, and cell survival. Hopefully, the system will provide leads toward more effecitve chemotherapy in lung cancer patients.

Preclinical studies have demonstrated synergism between the antidiabetic medication phenformin and BCNU in solid leukemia L1210.

Key Words:

--- Biocehmical pharmacology of new anticancer agents
--- Pharmacokinetics of new anticancer agents
--- Computer programs for pharmacokinetic analysis
--- Spin filter systems for combination chemotherapy
--- Preclinical study of phenformin plus BCNU

Biochemical Pharmacology Laboratory

Pharmacokinetic Modelling of Methotrexate:

The pharmacokinetics of methotrexate has become a subject of renewed interest with the use of high dose methotrexate with the leukovorin rescue. In particular, the importance of the terminal phase of plasma radioactivity after administration of plasma methotrexate has becoma a subject of considerable interest and controversy in so far as its clinical significance is concerned. For this reason we have studied plasma radioactivity after moderate and high dose methotrexate labelled with tritium and have collaborated with the laboratory of theoretical biology at NCI to carry out pharmacokinetic modelling. The analysis conducted by Drs. Berman and Leme using the program SAAM on a Univac 1108 computer (the program developed by Dr. Berman for pharmacokinetic modelling). The analysis shows that the plasma radioactivity consists of 2 distinct moieties and that the long half-life component is distinct form methotrexate. This finding which is clearly of very great clinical significance is the first demonstration that the long half-life after giving methotrexate is not methotrexate itself, and therefore, may be unimportant in so far as the toxicity of methotrexate is concerned. These studies are continuing with an analysis of methotrexate in laminar air flow isolation which is described as a separate project.

Pharmacokinetic Modelling of Methotrexate in Patients in Laminar Air Flow Isolation:

As part of our studies of methotrexate absorption in patients receiving intensive oral antibiotic treatment as part of the laminar air flow isolation regimen we have studied the plasma decay of radioactivity following oral tritium labelled methotrexate in patients both before and during administration of intensive antibiotic treatment in the laminar air flow room. Patients received 15 mg/m^2 of methotrexate labelled with 250 microcuries of tritium labelled material and the post-absorptive plasma decay of radioactivity was measured. Before antibiotic treatment, the plasma deacy showed the 2 phases a fast and a slow phase which has been described by ourselves and by others, however, during antibiotic treatment the show phase disappeared and the plasma decay was best described by a monoexponential decay. These results clearly collaborate the results obtained with the studies on high dose methotrexate in indicating that the slow half-life material is probably not methotrexate and is probably a product of gut bacteria which is not produced when these bacteria are destroyed. The project is continuing with a study of the pharmacokinetic of IV methotrexate in these patients.

Clinical Pharmacology of Thalicarpine:

In the course of the Phase I study of thalicarpine, the plasma decay and urinary excretion was studied in 20 patients. These studies have been carried out because there was no information on the clinical pharmacology of this drug and virtually no information on its pharmacology in animals. Doses of 300-1400 mg/m^2 of body surface area were investigated using 250 microcuries of thalicarpine specifically labelled with tritium in the 6 α-7 positions of the aporphine ring. Urinary recovery of the drug was erratic, very show, and incomplete. In 12 patients a total of 14 ± 6.1% was recovered in 72 h., and in 8 patients a total of 20.4 ± 9.4% was recovered in 10 days of total urine collection. The maximum recovery in any patient was 51.5% of the dose over a total of 65 days of total urine collection. Only trivial amounts of drug were recovered in the feces. The fate of the remaining drug is unknown but from the very slow excretion it would appear that the drug is being retained for a large extent in the body. In the first 6 days urine at the 1400 mg/m^2 the urinary radioactivity was 60% unchanged drug, 30% of metabolite, and 10% of volatile radioactivity which was probably radioactive water. As the tritium labelled thalicarpine was stable when incubated with plasma at 37° we concluded that the elimination of radioactivity as radioactive water was a result of a metabolic process and was not simply an exchange of labile tritium. Plasma decay of unchanged thalicarpine was triexponential. The terminal phase of the plasma half-life was very long with a mean half-life in 4 patients of 1400 mg/m^2 of about 14 days. There was no measurable thalicarpine in the cerebrospinal fluid.

These results are of considerable interest because they indicate a very long body retention of this drug. There was no indication from complete clinical parameters that this drug would have a long half-life; in fact, a few reports that exist indicate rapid plasma disappearance of the material. These findings emphasis once again the importance of carrying out clinical pharmacology studies early in the investigation of a new drug as the administration of this material on a daily schedule could easily lead to dangerous drug accumulation.

Clinical Pharmacology of VM26:

The study of the clinical pharmacology of VM26 [4'-demethylepipodophyllotoxin 9-(4,6-O-thenylidene-β-D-glucopyranoside)] has been completed. A total of 7 patients were studied with 250 microcuries of specific tritium labelled VM26 and a dose of 67 mg/m^2 body surface area of VM26 intravenously1 The principal findings were as follows: the drug resembled the chemically closely related VP16 in being excreted to about 50% in the urine. However, in marked contrast to the findings with VP16, the bulk of the urinary material in the case of

VM26 was metabolite (75%) whereas in the case of VP16 it was mostly unchanged drug. Recovery in the feces was erratic and was a maximum of 10%. The remainder of the drug is so far unaccounted for. Again in marked contrast to the findings with VP16, plasma decay was triexponential by non-linear least squares regression on an IBM computer. The half-life of the terminal phase of plasma decay ranged from 11-38 h. significantly longer than that of VP16. The drug did not penetrate into the cerebrospinal fluid to any measurable extent except in one patient; however, this patient had had extensive prior brain surgery and, therefore, cannot be considered to be a normal case. All patients evaluable for toxicity developed leucopenia (WBC < 5000/cu.mm) and 2 of the evaluable patients one with a laryngeal carcinoma and one with histiocytic lymphoma had some degree of clinical benefit from treatment with the drug. The major points of interest in this study are the marked contrasts between the pharmacology of VM26 and that of the very closely related VP16 which are being further investigated by pharmacokinetic analysis as a separate project, and the failure of this drug to cross the normal blood-brain barrier in spite of reports that the drug is active against brain tumors. It is not proposed to study this drug further as a single agent but it is proposed to study it in combination as it appears from a number of points of view to be a drug of considerable interest.

Pharmacokinetics of VM26 in Man:

Computer assisted analysis of the pharmacokinetics of VM26 in humans and comparison of the parameters with those for VP16-213, the methyl-congener. It was found that the plasma decay kinetics conformed best to a three-compartment open-model with first-order excretion and metabolism. The less than quantitative recovery of drug was conjectured to be due to sequestering of metabolite in its distribution space. The 1/6 smaller equitoxic dose compared to VP16 was related to the differences in plasma clearance and the 1/6th smaller renal clearance. The drug was found to bound to HSA to about 99%. The bioavailabiltiy was foudn to be superior with VM26 in one of its peripheral compartments compared to VP16. The study was completed in March, 1975.

Pharmacokinetics of VP16-213 in Humans:

Mathematical with computer assisted analysis of the plasma decay kinetics of VP16-213 in man, it was found that VP16-213 plasma decay conformed best to a two-compartment open-model with first-order excretion and metabolism. The inability to recovery 100% of administered dose was due to sequestering of metabolites in its distribution space. The 6 x greater equitoxic dose of VP16 over VM26 was accounted for in part by the nearly 6 x larger renal clearance and plasma clearance for VP16. Furthermore, the drug was about 94% protein bound in plasma. The study was completed in March, 1975.

Isolation and Identification of Human Metabolites of VM26 and VP16:

Methods of solvent, column, and thin layer chromatography have been used to carry out isolation and purification of the human urinary metabolites of VP16 and VM26 chemical derivatives for mass spectral analysis have been prepared for VP16 and VM26 and a synthetically derived "metabolite-like" adduct of VM26 and VP16 used as models for identification of mass fragmentation patterns of these complex organic molecules. Work is continuing with anticipation of bioassay evaluation of the metabolites for comparison to parent compounds.

Effect of VP16 and VM26 on Ray Liver Metabolizing Enzymes:

Initiated in November, 1974, a study is being conducted of the effect of chronic administration of VP16 and VM26 on levels of RNA, DNA, and protein in isolated rat liver by: assays on level of cytochrome P-450, aniline hydroxylase and N-demethylases in washed microsomes; kinetic analysis of drug metabolizing enzymes; and, evaluation of importance of dioxalone-function in VP16 and VM26 molecule as a general inhibitor of drug oxidation mechanisms.

Development Multicompartment Pharmacokinetic Model for VP16 and its Metabolite in Man:

A study was begun in February, 1975, to develop a mathematical model with computer assistance of VP16 and its metabolite in human plasma and urine. A complex model has been completed, demonstrating distribution and sequestering of drug in a peripheral space with some renal reabsorption of metabolite into plasma. Refinement of model continues.

Development of Multi-Dose Pharmacokinetic Profile Program, Pharmacodynamics Computer Sinulation Problems:

The project was initiated in October, 1974, for development of computer programs for pharmacokinetic analysis support, multi-dose kinetic profile predictions, development of pharmacokinetic cell response models (pharmacodynamic models for pharmacokinetics) for evaluation of new dosing regimens. Computer assessments of drug structure-biological activity relationships continues.

Biochemical Pharmacology of VM26 and VP16 in vitro:

Assays for extent of protein binding to human serum albumin in vitro via equilibrium dialysis were initiated in October, 1974. Effect on essential enzymes in mitochondria and nuclei (enzymes kinetics), are studied and new mechanisms of action using human peripheral lymphocytes-membrane effects are explored. Assays of DNA binding and its mechanism are performed.

Prediction of Potential Useful Drug Combinations Using the Isobologram and Development of the Rationale Based on Macromolecular Biosynthesis:

The study, begun in September 1974, includes assay of synergism, antagonism, or additive response of cultured L1210 cells to new Phase I chemotherapy agents in two agent combination using viable cell counts and isobolograms, development of biochemical rationale for isobologram profile using the nucleoside and nucleotide intermediates, and development of computer simulation of isobologram. Study continues.

Clinical Pharmacology of Isophosphamide:

The clinical pharmacology and pharmacokinetic parameters of a daily x 3 divided dose regimen of isophosphamide were investigated. Four selected patients were given isophosphamide at 1600 mg/m^2/day, or 2400 mg/m^2/day, for three successive days, as a 30 minute infusion, to which was added 10 microcuries of C-14 labelled drug. Appropriate plasma and urine samples were collected. Pharmacokinetic modelling was performed using a computer-programmed Gauss-Newton approach of linearizing the kinetic equations and applying linear least squares to determine parameters.

In contrast to the 2-compartment model observed with high single doses (up to 5,000 mg/m^2), the lower divided dose regimen best fitted a one-compartment model with monoexponential decay for unchanged drug. The plasma half-life for unchanged drug was 7.2 h. Approximately 90% of the dose administered was metabolized, in contrast to approximately 55% observed with the high single dose regimen. Urinary recovery of the total administered radioactivity was 55%, of which 15% represented unchanged drug. Current efforts to define a mathematical model for metabolite generation and distribution are under way.

Cell Culture Laboratory

Cell Culture and Drug Effects on an HCG Producing Cell Line (Chago) *In Vitro*:

Experiments are being accomplished with the Chago cell line, an HCG producing human cell line originally obtained from a patient with large cell anaplastic carcinoma of the lung. Data to date indicates a concordance between cell count and HCG production when treated with a variety of anti-neoplastic agents. The agents utilized include actinomycin-D, procarbazine, nitrogen mustard, methotrexate and vincristine. Initial data also indicate an interaction with certain of the agents producing an initial stimulation of cell growth in the presence of trypsin. The cell line has recently been adapted to spinner culture propagation and further work will be carried out not only in conventional suspension culture but also in the spin filter apparatus. Other area of investigation include cell kinetic analysis in both monolayer and spinner and also the effect of drugs on HCG production in spin filter culture. Hopefully this cell line may be useful as a model system for developing new drug combinations for human lung cancer.

Optimal Conditions for Combination Chemotherapy in Spin Filter Systems:

Experiments in the spin-filter apparatus were recently revised to insure adequate sterility, appropriate growth characteristics, and adequate support equipment. RPMI 1640 media is being used exclusively. Further experiments are being carried out to determine the effects of varying concentrations of fetal calf serum on growth rate and maximum cell concentration obtained. Further experiments are in progress to determine the amount of media utilization by cells at varying cell densities. Previous problems with contamination, equipment failure, and poor growth characteristics have been overcome. Four continuous flow experiments have been done in succession without serious problems. Growth rate characteristics in three have indicated doubling times between 26.4 and 33.6 h. at cell counts between 2 x 10^6/ml and 2.0 x 10^7/ml

Initial pharmacokinetic experiments have been accomplished utilizing methotrexate in the spin filter. Percent inhibitions have been similar to that obtained in conventional suspension culture for continuous treatment with somewhat greater percent inhibition with bolus administration. Additional data is being obtained in both murine leukemia L1210 as well as in the human tumor cell line, Chago.

Preclinical Studies

Phenformin (DBI) + BCNU:

Rationale: DBI is a mitochondrial poison that accumulates preferentially in acidic environments and, therefore, should concentrate in solid tumors.

Results: DBI in optimal schedule with BCNU gives 29% cures of solid L1210 vs. 5% cures for BCNU alone.

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Serial No.: Z01-CM-03403-10-M
1. Medicine Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Trials and Miscellaneous Clinical Investigations

Previous Serial Number: NCI-3403

Principal Investigators: Vincent T. DeVita, George P. Canellos, Robert C. Young, Bruce A. Chabner, Richard A. Bender, Philip S. Schein, Stephen H. Rosenoff, Susan P. Hubbard

Other Investigators: Ralph Johnson, William Terry, Harmar Brereton, Charles Myers, Richard Sherins, Bonny Johnson, First Year Clinical Associates, Medicine Branch, NCI

Cooperating Units: Radiation Branch, NCI, Immunology Branch,NCI, Reproduction Research Branch, NICHD

Man Years:

Total:	14.6
Professional:	12
Others:	2.6

Project Description:

The Medicine Branch is a major clinical facility of the National Cancer Institute. Its activities are divided between clinical therapeutic trials in patients with cancer, most of which are reported under project number Z01-CM-03403-10-M, and Project Report Z01-CM-06700-03-M Clinical Program in Breast Carcinoma, and clinical research efforts aimed at understanding the natural history of the disease in question, the interaction of the drugs used to treat cancer, the parallel effects of therapy on the host and the tumor, and techniques for earlier diagnosis and better follow up. These latter studies are reported in project reports entitled Clinical Pharmacology, Kinetics of Cellular Proliferation, Hematology Studies, Immunological Aspects of Cancer, Factors Controlling Lymphocyte Reactivity *In Vitro* and Steroid Receptor Studies in Malignancy.

Within the past year, a merger of the Medical Breast Cancer Service and the Medicine Branch has been accomplished with the result that the expanded Medicine Branch responsibilities now encompass all areas of adult medical oncology on the NIH reservation.

The clinical studies are conducted by the 7.5 senior staff (one investigator - Dr. Bruce Chabner from the Laboratory of Chemical Pharmacology - spends 50% of his time with the Medicine Branch), three research nurses and nine first-year Clinical Associates.

The clinical resources of the combined services now include 26 beds and 4 outpatient afternoon clinics a week. The volume of patients studied in the Branch and the variety of diseases treated have continued to increase significantly over the past year. The total outpatient census now approaches 700 patients and the majority of all therapy is administered in the out-patient clinics. The Medicine Branch has over the past several years made an increasing commitment to a greater variety of common solid tumors.

A. Clinical Trials with newer experimental drugs

1. Hexamethylmelamine

A Phase II trial of this agent in patients with alkylating agent resistant ovarian carcinoma is underway. Thirteen patients have been treated with an overall response rate of 38.5%. Toxicity is primarily gastrointestinal, neurologic and hematologic. Hexamethylmelamine continues to be one of the most active agents against ovarian cancer.

2. Bleomycin

A Phase II study of high dose intermittent Bleomycin has been completed. Although responses in lymphomas and testicular carcinoma were seen, the toxicity of the high dose intermittent regimen is excessive and produces no enhancement of chemotherapeutic effect.

3. Cytembena

A Phase I and II study of this agent in Breast and Ovarian Carcinoma is underway. Eleven patients (6 ovarian and 5 breast) have been entered thus far. Only one subjective response has been seen. Doses recommended from the Phase 1 trials done at other institutions have been associated with an induced renal tubular acidosis and proteinurea.

4. Gallium Nitrate

A Phase I trial of this unique antitumor agent is in progress. Thirteen patients have been entered. Doses have been escalated to 30 mg/M^2 I.V. weekly. No responses and no toxicity have been observed. Dose escalation will continue.

5. Epidodophyllotoxin (VP-16)

A Phase II trial in the treatment of drug-resistant histiocytic lymphoma has been initiated this year. Recent European studies suggest considerable activity of this structurally unique group of compounds in the lymphomas. VP-16 has been particularly active in histiocytic lymphoma, a very resistant, rapidly aggressive malignancy.

6. Achromycin (NSC403169)

Eighteen patients have received 100-675 mg/M^2 for an average of 30 days. The toxicity is primarily gastrointestinal with nausea and vomiting in 13 cases and mild thrombocytopenia in five cases. Two antitumor responses were noted - one myeloma and one soft tissue sarcoma.

7. 5-Azacytidine

This agent is being studied in patients with blastic transformation of Chronic Myelogenous Leukemia after failure on more standard agents for the disease. Four patients have been treated with 150 mg/M^2 qd x 5 days I.V. Thus far there have been two partial responders in the treated group.

B. Disease Oriented Studies

The following ten diseases are those most extensively studied by the Medicine Branch:

1. Histiocytic Lymphomas
2. Lymphocytic Lymphomas
3. Ovarian Carcinoma
4. Breast Carcinoma
5. Hodgkin's Disease
6. Osteogenic Sarcoma
7. Melanoma
8. Chronic Myelogenous Leukemia
9. American Burkitt's Lymphoma
10. Pancreatic Carcinoma.

1. Histiocytic Lymphoma

Long term results of the intensive chemotherapy of this highly resistant malignancy with MOPP and C-MOPP have been published this year and indicate that approximately 40% of patients with advanced disease will enter a complete remission and most of those completely responding will remain free of disease on no further therapy for periods of time up to nine years. These long term follow-up findings add Histiocytic Lymphoma to the group of malignancies in which long term disease irradiation with chemotherapy is possible.

2. Lymphocytic Lymphoma

The patterns of relapse after complete remission with CVP and C-MOPP combination have been reviewed. Patients with nodular poorly differentiated lymphocytic and diffuse well-differentiated lymphomas have demonstrated a pattern of continuous late recurrence. Initial sites of relapse are previously involved lymph nodes or bone marrow. Visceral sites not previously involved are rarely the initial site of relapse.

The Randomized Trial between Total Body Irradiation and Chemotherapy in the Non-Hodgkin's Lymphomas has been completed for most histologic groups and has been submitted for publication this year. The ongoing trial now compares combination chemotherapy with total body irradiation plus appropriate chemotherapy with CVP or C-MOPP.

A Prospective Trial of involved field radiotherapy compared with radiotherapy followed by C-MOPP therapy for localized Stage I and II histiocytic or mixed lymphomas continues. Combined modality therapy for extranodal Stage II diffuse histiocytic and mixed lymphomas continues.

3. Ovarian Carcinoma

A large scale study of ovarian cancer, the commonest fatal gynecologic malignancy, has been underway for three years combining in-house, contract, and laboratory based investigators. A randomized prospective trial comparing Melphalan with a 4 drug combination (Hexa-CAF), including Hexamethylmelamine, Cyclophosphamide, Methotrexate and 5-Fluorouracil continues. Preliminary results indicate an overall response rate for the combination of 85% (31%CR) compared to 60% (20%CR) for Melphalan.

In collaboration with the Radiation Branch, NCI, a prospective randomized study of Stage I and II patients is underway.

Other in-house studies include a) Phase II efforts with Hexamethylmelamine, Cytembena, or Adriamycin in patients with refractory ovarian carcinoma; b) Kinetic studies on patients with ovarian ascites tumor; c) Peritoneoscopy in the staging of ovarian cancer; d) determination of progesterone receptors in ovarian tumor tissue; 3) evaluation of lymphangiography in staging; f) determination of the clinical significance of tumor markers (CEA, HCG, Regan Isoenzyme, Serum Fucose, etc.) in ovarian cancer.

The Medicine Branch has continued its contractural arrangement with M.D. Anderson Hospital to identify new drug activity in ovarian cancer. The initial study identified Hexamethylmelamine as one of the most active single agents. The present study is comparing Melphalan, Hexamethylmelamine, Adriamycin and a combination of Cyclophosphamide-Hexamethylmelamine in patients with advanced previously untreated ovarian cancer. In addition a contract program initiated with the Mt. Sinai School of Medicine prospectively tested Thio-Tepa and Methotrexate and found Thio-Tepa superior.

4. Hodgkin's Disease

Most of the studies with Hodgkin's Disease have been completed and we are now summarizing our 10 year experience with combination chemotherapy in advanced disease on over 250 patients. In addition, a new trial in Stages IIB, IIIB, and IV disease is underway combining MOPP chemotherapy with Involved Field radiotherapy to sites of massive nodal disease. This trial was initiated because of a review of the patterns of relapse from complete remission after MOPP. Relapses are statistically more frequent in those patients with Nodular Sclerosis. Eighty-eight percent of patients relapsed in sites of previous involvement and 82% in sites of previous nodal involvement No patient relapsed exclusively in a visceral area not previously involved.

A new combination chemotherapy regimen (BAB) is being studied in patients failing MOPP. It employs three drugs (Bleomycin, Adriamycin and BCNU) not mutually cross reactive to those in the MOPP combination.

5. Osteogenic Sarcoma

A prospective adjuvant chemotherapy trial is underway comparing high-dose Methotrexate with high-dose Methotrexate and Adriamycin in patients after primary surgery. In conjunction with the clinical trial, detailed pharmacokinetic studies of high-dose Methotrexate are being completed in conjunction with the Laboratory of Clinical Pharmacology.

6. Breast Carcinoma

In addition to the studies summarized under Clinical Program in Breast Carcinoma Z01-CM-06700-03-M, follow-up studies on a CMF-(P) regimen previously reported by the Medicine Branch havebeen completed on 40 patients. The overall response rate is 68% with 20% complete remissions. The median duration of initial remission is 8 months.

7. American Burkitt's Lymphoma

A clinicopathologic study of 30 cases of American Burkitt's Lymphoma has been reported this year, with analysis of the important clinical features predicting prolonged survival after chemotherapy. In addition, an analysis of the sites of relapse and pathologic correlations has been published.

8. Melanoma

A trial comparing adjuvant immunotherapy or chemotherapy to no further therapy after complete resection in Stage II Melanoma is underway in conjunction with the Surgery Branch and Immunotherapy Branch, NCI. The Immunotherapy regimen employs BCG and autologous-cultured-neurominidase treated melanoma cells. The chemotherapy adjuvant is MeCCNU.

C. Clinical Investigations

1. Staging in the Non-Hodgkin's Lymphomas

A comprehensive staging study on approximately 200 patients jointly staged by the Medicine and Radiation Branches is nearing completion. In our series 74% of patients had Stage IV disease at completion of staging. Liver involvement and bone marrow involvement were the most frequent sites of visceral involvement.

2. Peritoneoscopy

Over the past several years the Medicine Branch has had an active program utilizing Peritoneoscopy in the staging and follow-up of patients with malignant diseases. Major efforts in its application in Hodgkin's Disease, non-Hodgkin's Lymphoma and Ovarian Cancer have yielded improved staging techniques and have provided an effective alternative to laparotomy.

3. Study of the Reproductive Capacity of Men and Women after Successful Intensive Combination Chemotherapy

Studies have been completed on males which indicate that a significant number (75%) treated with MOPP are sterilized by this therapy although some late recovery has been seen.

Reproductive function is being evaluated in 19 women who have been previously treated with intensive chemotherapy. Initial information indicates significant ovarian dysfunction in a significant proportion of women so treated.

4. Clinical Oncology Chemotherapy Nurse Program

Testing newly developed therapies has placed increased demands on medical oncology units. In a 6 months period, an average of 52 patients per clinic were seen. Thirty-eight percent (1146 patients) had I.V. chemotherapy by a chemotherapy research nurse. The use of chemotherapy research nurses improves delivery of care to cancer patients and opens a new area of specialization for nurses.

5. Second Tumors after Chemotherapy

Continuous monitoring of the incidence of second malignancies in patients who have received intensive chemotherapy and/or radiotherapy continues to be a major area of concern for the Medicine Branch. Continued follow-up on the 65 patients most highly at risk has revealed an incidence of second malignancies of 18 times normal. This increased incidence seems to be most associated with those patients receiving both chemotherapy and radiotherapy.

Keyword Descriptors:

1. Cancer Chemotherapy
2. Phase I & II Agents
3. Lymphomas
4. Ovarian Cancer
5. Breast Cancer
6. Peritoneoscopy
7. Cancer Staging
8. Second Malignancies
9. Chronic Leukemias
10. Hodgkin's Disease

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14. Banks, P.M., Arseneau, J.C., Gralnick, H.R., Canellos, G.P., DeVita, V.T. and Berard, C.W. American Burkitt's Lymphoma: A Clinicopathologic Study of 30 Cases. II. Pathologic Correlations. Am. J. Med. 58: 322-329, 1975.

15. Arseneau, J.C., Canellos, G.P., DeVita, V.T. and Sherins, R. J. Recently Recognized Complications of Cancer Chemotherapy. Ann. N.Y. Acad. Sci. 230: 481-488, 1974.

16. Schein, P.S. and Winokur, S.H. Immunosuppressive and Cytotoxic Chemotherapy: Long-Term Complications. Ann. Int. Med. 82: 84-95, 1975.

Serial No. Z01-CM-03404-05-M
1. Medicine Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immunologic Aspects of Cancer

Previous Serial Number: NCI-3404

Principal Investigator: Robert C. Young, Vincent T. DeVita, Stephen H. Rosenoff

Other Investigators: Richard I. Fisher, John E. Bennett, Michael Blaese, Harmar Brereton

Cooperating Units: Clinical Investigations Branch, NIAID, Immunology and Radiation Branches, NCI

Man Years:

Total:	0.5
Professional:	0.5
Others:	0

Project Description:

1. To study the immunologic status of patients with malignancies and relate these findings to progression or arrest of the disease and survival.

2. To exploit the hosts immunologic system as an adjuvant to chemotherapy of tumors.

3. To exploit antigenic markers in the therapy of patients with cancer.

4. To identify, study, and develop therapy for infectious complications of patients with cancer.

A. Immunology and Cancer

1. Immunologic studies in Non-Hodgkin's Lymphoma (VII,7XX,SA25,EA75)

Use of serum and skin testing to define the immunologic defect in Non-Hodgkin's lymphoma is underway. The immunologic rebound after chemotherapy is also being studied. The prognostic significance of cellular and humoral immunity in patients is being analyzed prospectively. A prospective response to therapy is being conducted in all histologic forms of non-Hodgkin's lymphoma.

2. Immunologic Studies in Hodgkin's Disease (VVI,7XX,EA100)

A re-evaluation of the immune status of patients who enter complete remission is now underway and includes retesting patients for delayed hypersensitivity after they achieve remission. Evidence accumulated thus far suggests that the immunologic defect present with Hodgkin's disease has in some instances been eliminated by successful chemotherapy.

3. Tumor Specific Antigen in Ovarian Carcinoma (V,5XX,LA100)

Attempts are being made to grow human ovarian ascites tumor in cell culture to obtain antigens for high titer specific antibody and to assay for hormone receptor binding.

4. Tumor Marker Analyses in Ovarian Carcinoma (V,6X1,SA50,EA50)

In cooperation with Dr. Phillip Waalkes' laboratory we have supplied serum, ascites and urine from patients with ovarian cancer to assay for the prospective study of CEA, HCG, Regan Isoenzyme, Serum Fucose, and Polyamines, as markers for tumor burden, response to therapy and time of relapse.

B. Infections and Cancer

1. Candidiasis (VI,6X1,SA50,EA50)

A comprehensive review of the clinical pathologic characteristics of disseminated candida infections in 250 patients with malignancy is being completed.

2. Fungemia (VI,6X1,SA50,EA50)

An analysis of 70 patients with fungal sepsis was completed and criteria for establishing the significance of antemortem fungenia were published.

3. Pneumocystis Carinii (VI,6X1,SA50,EA50)

A symposium on Pneumocystis Carinii - diagnosis, prognosis, treatment and results was held this year and will be published in monograph form in the Journal of the National Cancer Institute. The current therapy program involves a randomized trial comparing Pentamidine with Pyrimethamine and Sulfadiazine.

Keyword Descriptors:

1. Immunotherapy
2. Delayed Hypersensitivity
3. Immunosuppression
4. Tumor Markers
5. Tumor Associated Antigens
6. Fungal Infection
7. Immunosuppressed Host
8. Pneumocystis
9. Infection in Malignancy

Publications:

1. Young, R.C., Bennett, J.E., Geelhoed, G.W. and Levine, A.S.: Fungemia with Compromised Host Resistance. *Ann. Int. Med.* 80: 605612, 1974.

2. DeVita, V.T., Goodell, B., Hubbard, S., Geelhoed, G.W. and Young, R.C.: Pneumocystis Pneumonia in Patients with Cancer: Clinical Setting. *J.N.C.I.* (in press).

3. Young, R.C. and DeVita, V.T.: The Treatment of Pneumocystis Carinii Pneumonia: Current Status of the Regimens of Pentamidine Isethionate and Pyrimethamine - Sulfadiazine. *J.N.C.I.* (in press).

4. Levine, A.S., Schimpf, S.C., Graw, R.G. and Young, R.C.: Infections in Malignant Disease States: Progress in Management. *Seminars In Hematology* 11: 141-202, 1974.

5. Levine, A.S., Schimpf, S.C., Graw, R.G. and Young R.C.: Hematologic Malignancies and Other Marrow Failure States: Progress in the Management of Complicating Infections. *Lymphoma and Leukemia*. Edited by Fairley, G.H., Jaffe, E.R. and Miescher, P.A. Grune and Stratton, Inc. 1974.

Project No. Z01-CM-03405-06-M
1. Medicine Branch
2. Hematology Investigation
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hematology Investigations

Previous Serial Number: NCI-3405

Principal Investigators: George P. Canellos, Vincent T. DeVita, Jr., Jacqueline Whang-Peng, Philip S. Schein, Bruce A. Chabner, Robert C. Young

Other Investigators: Louis Gelrud, Gerald Johnston

Cooperating Units: Surgery Branch, NCI; Cytogenetics Section, NCI; Nuclear Medicine Dept., Clinical Center, NIH; Laboratory of Clinical Investigation, NIAID

Man Years:

Total:	1.6
Professional:	1.2
Other:	0.4

Project Description:

Clinical and laboratory investigations in patients with chronic myelogenous leukemia have been facilitated by the fact that patients with this disorder have (1) abundant granulocytes for periods of time off treatment, (2) undergoes a transition from a premalignant to a highly malignant disease as part of its natural history. In the past year, efforts have been primarily directed toward using the granulocyte of this disease as a human tissue source for the study of a new granulocyte-labelling isotope, ^{67}Gallium.

A. Cytogenetic studies (V 5XX, LA25, SA50, EA25)

Collaboration with Dr. J. Whang-Peng has continued in an attempt to define a correlation between cytogenetic findings during blastic transformation and response to specific therapy. This represents the most completely studied groups of patients from diagnosis through to blastic crisis. Some of the more significant correlations are included under the discussion of blastic crisis therapy.

B. Therapeutic trials - CML

1. Chronic Phase Chemotherapy (VI, 6X1, SA50, EA50)

Forty patients have been randomized between dibromomannitol (DBM) and busulfan in order to investigate the comparative effectiveness of the former drug, a relatively new agent, in a group of previously untreated, Philadelphia chromosome positive recently diagnosed patients. The study has been completed and has required 5 years to properly evaluate this new agent in CGL. There were 16/20 complete remissions with DBM and 14/20 with busulfan. Failures were due to early blastic crisis and severe hypoplasia in 2 cases treated with busulfan. Mean time to remission was shorter with DBM (72 days) vs. 100 days for bufulfan. Busulfan resulted in a more prolonged myelosuppressive effect such that 9/14 remitters required a median time of 9 months before drug could be readministered for maintenance compared with only 3/16 DBM patients who required a delay. Extensive histologic evaluation revealed no differences in the extent of cellular atypia produced in lung or bladder tissue. The survival for the groups did not significantly differ.

2. Splenectomy (VII, 7XX, EA100)

The role of splenectomy on the natural history of chronic granulocytic leukemia is being investigated. Thus far 24 patients have had prophylactic splenectomy. Thirteen patients have entered blastic transformation and twelve of these received chemotherapy. The median survival of this latter group is 6 months (range 3-10), despite absence of complete remission. The general supportive care of such patients was felt to have been markedly improved. There was only one complication of the splenectomy--postoperative bleeding in a single case.

3. Treatment of Blastic Phase of Chronic Granulocytic Leukemia (VI, 6X1, LA 100)

Since the demonstration that vincristine and prednisone were capable of inducing hematologic remission in 30% of a series of 30 consecutive patients with blastic crisis of CGL, a randomized trial of this combination versus arabinosyl cytosine (ARA-C) (200 mg/M^2) and 6-thioguanine (200 mg/M^2) was begun. To date 32 patients have been studied. Thirteen have received ARA-C/6TG with one complete and 3 good partial responses. Nineteen have received vincristine, prednisone with 2 complete remitters in the latter groups has presented with hypodiploidy (44 Ph'). Including cases from our previous study 7/10 patients who presented with hypodiploidy at time of blastic crisis achieved a complete or partial hematologic remission. Crossover to opposite drug regimen was attempted in 22 cases with a partial response in 3 cases.

C. Laboratory Investigations

1. The radionuclide ^{67}Ga has been extensively investigated. In summary, studies to date, some of which have been published, have revealed marked affinity of normal and leukemic granulocytes for the isotope. Further, this has been correlated with the presence of lysosomes. In addition, lymphocytes under the influence of PHA were shown to increase their capacity to bind ^{67}Ga which is a function of the appearance of lysosomes after PHA. Mouse melanoma cells have been studied by subcellular fractionation density gradients with marked localization of ^{67}Ga to melanosomes. Thus, we may have discovered a specific marker isotope for these organelles.

2. Studies of Leukocyte NADH Oxidase (VI, 6X2, SA50, EA50)

Neutrophil NADH oxidase has been suggested as the primary enzyme involved in bacteriocidal activity, and deficient in patients with chronic granulomatous disease who are predisposed to infection. A sensitive micro assay system has been developed for the measurement of NADH oxidase in order to determine the effect of disease and chemotherapy on the enzyme's activity.

Publications:

1. Rosenoff, S.H., Canellos, G.P., O'Connell, M. and Wiernik, P.H.: Mediastinal Adenopathy in Granulocytic Leukemia. Arch. Int. Med. 134: 135-138, 1974.

2. Canellos, G.P.: Hypercalcemia in Malignant Lymphoma and Leukemia. Ann. N. Y. Acad. Sci. 230: 240-246, 1974.

3. Sponzo, R.W., Arseneau, J.C. and Canellos, G.P.: Procarbazine Induced Oxidative Hemolysis: Relationship to In Vivo Erythrocyte Survival. Brit. J. Haemat. 27: 587-595, 1974.

4. Mason, J.E., DeVita, V.T. and Canellos, G.P.: Thrombocytosis in Chronic Granulocytic Leukemia. Blood 44: 483-487, 1974.

5. Canellos, G.P., Young, R.C., Nieman, P.E. and DeVita, V.T.: Dibromomannitol in the Treatment of Chronic Granulocytic Leukemia: A Prospective Randomized Comparison with Busulfan. Blood 45: 197-203, 1975.

Keyword Descriptors: Chronic myelogenous leukemia, cytogenetic studies, dibromomannitol, prophylactic splenectomy, chronic granulocytic leukemia, vincristine, prednisone, radionuclide ^{67} Gallium, NADH oxidase.

Serial No. Z01-CM-03420-06-M
1. Medicine Branch
2. Cellular Kinetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Kinetics of Cellular Proliferation

Previous Serial Number: NCI-3420

Principal Investigators: Robert C. Young, Bruce A. Chabner, Stephen H. Rosenoff, Charles Myers, Vincent T. DeVita, Jr.

Other Investigators: Harmar Brereton, Daniel Ihde, William McGuire

Cooperating Units: Laboratory of Chemical Pharmacology, NCI
Radiation Branch, NCI

Man Years:

Total: 4.0
Professional: 2.0
Other: 2.0

Project Description:

The goals of the tumor cell kinetic program of the Medicine Branch over the past six years have been:

1. To examine and develop methodology that might be useful in obtaining cell kinetics information in individual patients in a time period that may allow application of these data to the patient's therapy.

2. To describe the kinetics of human tumors, compare them to animal models, and to compare results to normal tissues, to the same tumor in different patients, and to different metastatic sites of the same tumor in the same patients.

3. To examine the manner in which cell cycle characteristics are changed in response to exposure to drugs.

4. To approach the problem of manipulating tumor growth characteristics in a way to increase susceptibility to chemotherapeutic agents.

A. Kinetic Alterations induced by Chemotherapy in vivo

A series of studies have been completed on the alterations induced by Cyclophosphamide, BCNU, MeCCNU, Cytosine Arabinoside, Cyclocytidine, 5-azacytidine, and Adriamycin in normal and tumorous target tissues in vivo. All these agents show a differential sensitivity of the tumor compared to normal tissues and proper sequences of drug therapy can be designed around these kinetic differences. Both ascites tumor models (i.e. L1210,P388) and solid tumor systems (B16 Melanoma) have been studied.

B. Kinetic - Pharmacokinetic studies

In cooperation with the Laboratory of Chemical Pharmacology, NCI studies on the pharmacokinetic disposition of drugs such as Methotrexate and 5-FU have been compared to simultaneous kinetic alterations. Results of two such studies have been published this year.

C. Human Kinetic - Pharmacokinetic Studies

Studies on the effect of methotrexate on the suppression and recovery of DNA synthesis in human bone marrow and human ovarian carcinoma ascites cells have been completed in six patients with simultaneous study of blood and ascites concentration of methotrexate and clinical response. DNA synthesis in human tissues in vivo recovers when the concentration of methotrexate in the blood falls to 1x10^{-8}M or lower. Differential toxicity to normal and tumorous tissue can be used to predict clinical sensitivity or resistance to methotrexate in these patients with ovarian cancer.

D. Adriamycin Kinetic Studies

Studies over the past year have established the BDF^{1} mouse as another suitable model for acute and chronic adriamycin cardiotoxicity. Alterations in DNA synthesis, light microscopic, and election micrographic studies all demonstrate cardiomyopathy of the type seen in humans. This model is now being used for a screen for non-cardiotoxic adriamycin and daunomycin derivatives.

Keyword Descriptors:

1. Pharmaco-kinetics
2. Cellular Kinetics
3. Cell Cycle
4. DNA Synthesis
5. Adriamycin Cardiotoxicity

Publications:

1. Rosenoff, S.H., Bostick, F. and Young, R.C.: Alterations in DNA Synthesis Induced by BCNU in Normal and Tumorous Tissues: Implications for Optimal Chemotherapy. *Biochemical Pharm.* 23:3097-3106, 1974.

2. Myers, C.E., Young, R.C., Johns, D.G., Chabner, B.A.: Enzymatic Assay of Intracellular 5-Fluorodeoxyuridine 5-Monophosphate and Deoxyuridine-5-Monophosphate Following 5-Fluourouracil. *Cancer Res.* 34:2682-2688,1974.

3. Rosenoff, S.H., Bull, J.M. and Young, R.C. The Effect of Chemotherapy on the Kinetics and Proliferative Capacity of Normal and Tumorous Tissues *In Vivo*. *Blood* 45:107-118, 1975.

4. Rosenoff, S.H., Olson, H.M. and Young, R.C.: Alteration in DNA Synthesis in Cardiac Tissue and Light Microscopic and Ultrastructural Cardiac Damage Induced by Adriamycin *In Vivo*. *Second Int. Symposium on Adriamycin*. Brussels, Belgium. (in press.)

5. Rosenoff, S.H., Bostick, F., and Young, R.C. Recovery of Normal Hematopoietic Tissue and Tumor Following Chemotherapeutic Injury from Cyclophosphamide: Comparative Analysis of Biochemical and Clinical Techniques. *Blood* (in press).

6. Rosenoff, S.H., Brooks, E., Bostick, F. and Young, R.C.: Alterations in DNA Synthesis in Cardiac Muscle Induced by Adriamycin *In Vivo*: Relationship to Fatal Toxicity. *Biochem. Pharm.* (in press).

7. Myers, C.E., Young, R.C. and Chabner, B.S.: Biochemical Determinants of 5-Fluorouracil Response. The Role of Deoxyuridylate Pool Expansion. *J. Clin. Invest.* (in press.)

8. Rosenoff, S.H., Olson, H.M., Young, D.M., Bostick, F. and Young, R.C.: Adriamycin Induced Light Microscopic and Ultrastructural Cardiac Damage in the Mouse: A Small Animal Model of Cardiotoxicity. *J. Nat. Cancer Inst.* (in press)

9. Brereton, H.D., Bryant, T. and Young, R.C.: Inhibition and Recovery of DNA Synthesis in Host Tissues in Sensitive and Resistant B16 Melanoma After Methyl CCNU - A Predictor of Clinical Efficacy. *Cancer Res.* (in press)

Serial No.: Z01-CM-06500-03-M
1. Medicine Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: "Clinical Pharmacology -- Mechanism of Action, Physiologic Disposition, Metabolism, and Toxicity of Anticancer Agents"

Previous Serial Number: NCI-6500

Principal Investigators: Bruce Chabner, Richard Bender, Charles Myers, Robert Young

Other Investigators: Robert Diasio, John Bennett

Cooperating Units: Laboratory of Chemical Pharmacology and Toxicology, NCI, Diabetes Section, Endocrinology Branch, NIAMDD

Man Years:

Total:	4.5
Professional:	2.5
Other:	2.0

Project Description:

A significant area of interest in medical oncology is the identification and understanding of mechanisms of action of new anticancer agents. The Section of Clinical Pharmacology is also involved (1) in the design and conduct of new drug (Phase I) trials, (2) development of methodology for analysis of new and old chemotherapeutic agents and (3) conduct of studies of absorption, distribution, metabolism, and excretion of cancer chemotherapeutic agents. Laboratory studies are aimed at improving our understanding of antitumor and toxic drug effects that may lead to development of new agents with a higher therapeutic index.

A. Antifolate Pharmacology

1. Folate Transport Studies.

This laboratory has been interested in the membrane transport of methotrexate (MTX) in human neoplastic cells. Investigations in blast cells from approximately 15 patients with acute myelogenous leukemia, acute lymphocytic leukemia, and Burkitt's lymphoma suggest that MTX is transported into human neoplastic cells via an energy-dependent carrier mechanism at 10^{-6}M MTX concentrations. The carrier system appears saturable and has a K_m of about 6×10^{-6}M in ALL cells. Further, the observation that MTX and folinic acid compete for a common or closely-coupled carrier site for cellular entry has implications in the timing of "rescue" of MTX-poisoned cells. Additional studies have shown that the concomittant administration of other drugs to MTX-treated cells can inhibit or enhance the uptake of MTX by these cells. Hydrocortisone and Keflin both inhibit MTX uptake and Vincristine (VCR) enhances MTX uptake by retarding the exit of drug from the cell. Of importance, VCR increases exchangeable intracellular MTX in excess of that bound to dihydrofolate reductase, the principle site of MTX action and of intracellular binding. Increasing the exchangeable intracellular MTX has been shown to further inhibit the incorporation of deoxyuridine into DNA in this and other laboratories in a variety of animal cell lines. Present studies are designed to examine the role of exchangeable intracellular drug and of VCR in the cytotoxicity of MTX in human cell lines.

2. Methotrexate Assay

The currently available methods for measure of Methotrexate levels in biological fluids are time consuming and complex. Recent developments in the use of antifolates clinically have established important clinical correlations for Methotrexate levels in plasma, urine, and CSF, and have prompted us to investigate new methods. We have developed a competitive protein binding assay for MTX which is rapid (1-2 hrs), sensitive (10^{-9}M) and highly specific for titrating inhibitors of dehydrofolate reductase. The assay is based on competitive binding of known, labelled ^{3}H-MTX and an unknown sample of MTX to bacterial reductase, with separation of bound and free drug by charcoal absorption. This assay also allows precise determination of the binding affinity of antifolates to reductase for the first time, and should be useful in quantitating this interaction for new folate analogs.

3. Studies of the relationship of plasma and ascites pharmacokinetics to drug effect in patients with ovarian carcinoma.

These studies are described in detail in project reports (Young - Kinetics Section, Chabner - LCP report).

B. Studies with Fluorinated Pyrimidines

1. 5-Fluorouracil

Enzymatic assays for 5-FdUMP and dUMP have been developed and applied to the problem of determining the pharmacokinetic changes in these nucleotides associated with 5-FU administration in experimental animals and man. These changes have related the suppression and recovery of DNA synthesis in normal and malignant tissues in mice bearing the P1534 leukemia and have revealed that the duration of inhibition produced by 5FU is determined by the rate of disappearance of free FdUMP, as well as the rate of accumulation of the normal, competitive substrate, dUMP. Changes in these nucleotides were different in each of the tissues examined, but the ratio of nucleotides during recovery of DNA synthesis was remarkably consistent from one tissue to the next. A general model for the events determining suppression and recovery of DNA synthesis was proposed, in which accumulation of the competitive normal substrate plays a crucial role in preventing inactivation of newly synthesized thymidylate synthetase. Further studies are underway to examine these relationships in man.

2. Studies on the mechanism of action of 5-flurocytosine and agranulocytosis produced by this agent.

This antifungal agent is the subject of considerable clinical interest, but its metabolism and mechanism of action are poorly understood. Using Candida Albicans as a test organism, we are studying the metabolism of this agent, including its conversion to FdUMP by normal tissue and fungi, and its effect on the thymidylate synthetase pathway. In addition, we are examining these parameters in bone marrow samples from patients developing agranulocytosis while on this drug.

3. Ftorafur

This agent, a furanosyl derivative of 5-FU, is thought to act as a slow release form of 5FU, but little information exists at present as to its metabolism and mechanism of action in normal and neoplastic tissue. Preliminary studies in mice in our laboratory have demonstrated the synthesis of FdUMP and a selective inhibition of thymidylate synthetase in both bone marrow and tumor cells. The time course of these changes in comparison to 5-FU is currently under study.

Keyword Descriptors

1.	Methotrexate	4.	5-Fluorouracil
2.	Transport	5.	Protein Binding Assay
3.	Pharmacokinetics	6.	Myelosuppression

Publications:

1. Bender, R.A. Membrane transport of methotrexate in human neoplastic cells. Cancer Chemother. Rep. (in press).

2. Bender, R.A., Bleyer, W.A., Frisby, S.A., and Oliverio,V.T. Alteration of methotrexate uptake in Human Leukemia Cells by other Agents. Cancer Res. (in press).

3. Myers, C.E., Young, R.C., Johns, D.G. and Chabner, B.A. Assay of 5-Fluorodeoxyuridine 5'-Monophosphate and Deoxyuridine 5'-Monophosphate Pools Following 5-Fluorouracil. Cancer Res. 34:2682, 1974.

4. Myers, C.E., Young, R.C. and Chabner, B.A. Role of dUMP accumulation in Recovery from Inhibition of DNA Synthesis by 5-Fluorouracil. J. Clin Invest. (in press).

Project No. Z01 CM 06700-03 M
1. Medicine Branch
2. Medical Breast Cancer Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Program in Breast Carcinoma

Previous Serial Number: NCI-06700

Principle Investigators: Douglass C. Tormey, M.D., Ph.D., Joan M. Bull, M.D., Paul P. Carbone, M.D., Marc Lippman, M.D., and C.M. Myers, M.D.

Other Investigators: A.S. Ketcham, M.D., S. Rosenberg, M.D., E.V. de Moss, M.D., J. Blom, M.D., E. Perlin, M.D., G. Falkson, M.D., P. Band, M.D., T. Pomeroy, M.D., D. Henson, M.D., J. Doppman, M.D., E. Jones, M.D., G. Johnston, M.D., M. Cohen, M.D., Ph.D., P. Chretien, M.D., R. Herberman, M.D., R. Oldham, M.D., first year clinical associates, second year clinical associates, P. Schein, M.D., F. Chu, M.D., J. Whang-Peng, M.D., R. Knazek, M.D., R. Everson, M.D., B. Thompson, M.D., P. Waalkes, M.D., Ph.D., R. Simon, Ph.D., S. Carter, M.D., A. Barlock, A.N., C. Graw and contractors

Cooperating Units: Surgery Branch, NCI, Radiation Branch, NCI, Pathology Branch, NCI, Office of the Associate Director for MO, NCI, Cancer Therapy Evaluation Branch, NCI, contractor hospitals, Diagnostic Radiology, NCI, Nuclear Medicine, NCI, Laboratory of Pathology, NCI, Laboratory of Chemical Pharmacology, NCI, Laboratory of Biochemistry, NCI, Experimental Biology Projects Section, NCI, Tumor Cell Biology Laboratory, NCI, Cellular and Tumor Immunology Section, NCI, Therapeutic Projects Section, NCI, and the Epidemiology Branch, NCI

Man Years:

Total: 12.1
Professional: 8.50
Other: 3.6

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, MO, until its shift to the Medicine Branch in August 1974. The program is currently supported by one secretary, two technicians, one second year clinical associate, three first year clinical associates and, until August 1974, direct personnel support from the OAD, Nursing Service, Experimental Biology Projects Section, and Epidemiology Branch, NCI in order to support the clinical program. The program developed with an interdisciplinary approach to treatment with the Surgery Branch, Radiation Branch, and Pathology Laboratory at NCI. It grew from 4 to 7 beds on 13-East in July 1973. These were transferred to the Medicine Branch in August 1974. Four one-half day clinics were maintained, including a joint Breast Problem Clinic with the Surgery Branch. In December 1974 this latter clinic's activities were switched to the Surgery Branch. Bed utilization had approximated 100%. 4000 clinic visits were projected for this fiscal year.

I. Clinical Trials

A. Approach to the primarily diagnosed patient. 7XX

1. The Breast Problem Clinic was geared at evaluating tumor markers (See II.D) and other diagnostic techniques prior to biopsy. These patients are now seen by the Surgery Branch and the tumor marker evaluations continue.

2. Following radical mastectomy, patients with histopathologically positive axillary lymph nodes are entered into an NCI protocol comparing observation to cyclophosphamide, methotrexate, and 5-fluorouracil with/without C. parvum. L-PAM was substituted for observation in September 1974. 32 entries as of April 1975. It is too early for analysis.

B. Hormone Plus Chemotherapy Trials in Patients With A First Recurrence of Disease. 7XX

1. Between 5 and 15% of Stage I and II patients present with their first recurrence at the mastectomy site. These patients undergo an excisional biopsy to confirm the recurrence, and have been evaluated clinically to ascertain if there are metastatic foci; if there are they become candidates for studies listed below (2 and 3). If there is no clinical evidence of disease the patients then enter a protocol which compares oophorectomy to oophorectomy plus combination chemotherapy in premenopausal patients, or observation against combination chemotherapy in postmenopausal patients. 2 entries. A low acquisition rate led to discontinuation of protocol in 1974.

2. Premenopausal patients with a measurable first recurrence enter a protocol in which they undergo a bilateral oophorectomy followed by observation for up to 12 weeks. If progressive disease occurs in this interval they enter the second recurrence program (See C). If a response or no change status is present at 12 weeks, they are then randomized to receive either combination chemotherapy or no further therapy. The latter group will receive the chemotherapy regimen when progressive disease develops. 22 entries as of April 1975. Too early for analysis.

3. Postmenopausal patients with a measurable first recurrence enter a protocol in which they initially receive diethylstilbesterol for 12 weeks. If progressive disease occurs in this interval or no change occurs at 12 weeks, they are then randomized to receive either diethylstilbesterol alone or in combination with chemotherapy. The patients relapsing on the hormone alone arm will subsequently receive combination chemotherapy after progressive disease occurs. 20 entries as of April 1975. It is too early for analysis.

C. Second Recurrence Investigations. 6X1

1. A protocol comparing cyclophosphamide, methotrexate, and 5-fluorouracil to cyclophosphamide, adriamycin, and 5-fluorouracil was closed in October 1974. There were 88 entries. The adriamycin containing arm appears superior to the methotrexate containing arm ($p<.05$).

2. The protocol in 1 was replaced by a trial comparing cyclophosphamide, adriamycin and 5-fluorouracil to sequential therapy with combinations of adriamycin, dibromodulcitol, vincristine, and cyclophosphamide, methotrexate, 5-fluorouracil. 22 entries as of April 1975. Both regimens are well tolerated. Too early for analysis.

3. A Phase II protocol evaluating the combination of dibromodulcitol and adriamycin closed in August 1974 with 57 entries. All patients were previously exposed to chemotherapy. The response rate was 36%.

4. A study evaluating dibromodulcitol alone as intermittent therapy has accrued 17 patients. Response rate approximately 20%.

5. A protocol comparing the anti-estrogen Nafoxidine against Nafoxidine plus Fluoxymesterone was activated and revised to substitute Tamoxifen, a less toxic anti-estrogen, for Nafoxidine. 27 entries. Too early for analysis.

6. A Phase I evaluation of Lilly Compound 83636 for its prolactin suppressing capability was activated. The trial was temporarily suspended by the FDA after 4 patient entries due to side-effects encountered in another institution. The company anticipates that the drug will be available for further study.

7. A Phase II trial of BCNU and methotrexate was activated in August 1974. It is too early for analysis.

8. A Phase I-II trial of Cytembena was activated for breast and ovarian cancer in September 1974. 4 entries as of March 1975. Renal toxicity was encountered and is being evaluated.

II. Ancillary Studies

A. Chromosome Analysis

In cooperation with Dr. Jacqueline Whang-Peng and the Surgery Branch we are attempting to provide material serially on patients for chromosome analyses. This project is designed to further our understanding of the disease process and to evaluate the role of chemotherapy in the induction of chromosomal abnormalities.

B. Tumor Marker Analyses

In cooperation with Dr. Phillip Waalkes' laboratory we have supplied blood and urine samples from patients for analysis of tumor markers. The results of this program indicate that 65% to 70% of patients pre- and postoperatively, and 97% of patients with metastatic disease, will have elevations of one or more of the following markers: human chorionic gonadotrophin, carcinoembryonic antigen, or N^2, N^2-dimethylguanosine. We are continuing to evaluate new markers and are now evaluating the relationship of these markers to tumor burden, site of lesions, pathology, and response to therapy.

C. Radionuclide Scanning Procedures

The diagnostic importance of brain, liver-spleen, bone, and gallium scans in breast carcinoma had not been evaluated systematically. We established with the Radionuclide Branch a standard series of scans to be performed on a routine sequential basis on our patients. The results of these procedures are being correlated with the clinical state to ascertain their capabilities relative to other clinical modalities. At the present time the data suggests that brain and liver-spleen scanning are not very useful parameters and gallium scanning appears to be a good screening modality only for the detection of mediastinal lesions. Bone scanning is superior to roentgenographic procedures. Recommendations for the use of these procedures are being formulated.

D. Hormone Analyses

We have measured a variety of steroid hormones and hormone metabolities before and after chemotherapy in our patients. The results of these analyses are now back from the contracting laboratory and are available for analysis.

E. Evaluation of Steroid Binding Proteins

Dr. Brad Thompson was receiving tissue from the Surgery Branch and the Medical Breast Cancer Service for the analysis of steroid binding receptors. This project was transferred to Dr. Marc Lippman when he joined our staff in July 1974. A description of the programs can be found in his report.

III. Extramural Activities

The activities listed below have a direct bearing on the program of the Medical Breast Cancer Service in terms of either providing patient material or an integrating (breast cancer) program among various investigators.

A. Contract Programs 6X1, 7XX, 5XX

The National Naval Medical Center, Bethesda, and Walter Reed Army Hospital, Washington, are under contract to NCI. Douglass C. Tormey is project officer for these contracts. Both institutions take part in our protocol designs and utilization, thus shortening the duration of time required to complete our studies. To a lesser extent they participate in some of our ancillary study programs.

Dr. Geoffrey Falkson of Pretoria, South Africa and Dr. Pierre Band of Edmonton, Alberta both cooperate with the Medical Breast Cancer Service on a non-contractoral basis to participate in several of the chemotherapy studies.

B. Breast Cancer Task Force 1XX, 7XX

The Medical Breast Cancer Service participates in the Treatment Subcommittee of the Breast Cancer Task Force.

C. Cooperative Group Affiliations 5XX, 5X1, 7XX

The Medical Breast Cancer Service is affiliated with Acute Leukemia Group B, in which Douglass C. Tormey is study chairman of two breast cancer studies. Douglass C. Tormey is chairman of the Breast Tumor Subcommittee of the Eastern Cooperative Oncology Group, and is co-author of two Metastatic Disease Protocols in that group.

Key Descriptors:

breast cancer
adjuvant chemotherapy
adjuvant chemoimmunotherapy
chemotherapy
chemoimmunotherapy
hormones
clinical trials
hormonochemotherapy
data handling techniques
chromosomes
tumor markers

Publications:

1. Silverman, A.Y., Yagi, Y., Pressman, D., Ellison, R.R., and Tormey, D.C.: Monoclonal IgA and IgM in the Serum of a Single Patient (SC). III. Immunofluorescent Identification of Cells Producing IgA and IgM. J. Immunol. 110: 350-353, 1973.

2. Tormey, D.C.: Neurotoxicity of Vincristine. Lancet 1: 1312, 1973.

3. Stutz, F.H., Blom, J., and Tormey, D.C.: Combination Chemotherapy in Disseminated Carcinoma of the Breast. Oncology 29: 139-146, 1974.

4. Tormey, D.C., Gailani, S., and Leone, L.: Phase II Evaluation of a Combination of BCNU and 5-FU in Gastrointestinal Carcinomas. Oncology 29: 244-248, 1974.

5. Bergevin, P., Blom, J., and Tormey, D.C. eds.: Outline of Therapeutic Oncology with Emphasis on Cancer Chemotherapy. Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

6. Enquist, R.W., Tormey, D.C., Jenis, E.H., and Warkel, R.L.: Malignant Chemodectoma With Elevated Urinary Homovanillic Acid. Chest 66: 209-211, 1974.

7. Tormey, D.C. and Blom, J.: Chemotherapeutic Principles: Therapeutic Concepts, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

8. Tormey, D.C., and Chabner, B.A.: Classification of Chemotherapeutic Agents, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

9. Bergevin, P., Tormey, D.C., and Blom, J.: Chemotherapeutic Agents; Individual Drugs, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

10. Tormey, D.C.: Chemotherapeutic Principles: The Cell Cycle, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

11. Tormey, D.C.: Chemotherapeutic Principles; Cell Kinetic Considerations, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

12. Bergevin, P., Tormey, D.C., and Blom, J.: Chemotherapeutic Principles; Drug Dosage Guidelines, in Outline of Therapeutic Oncology; With Emphasis on Cancer Chemotherapy (Bergevin, P., Blom, J., and Tormey, D.C. eds.) Accepted for publication by University Park Press, Baltimore, Maryland, 1974.

13. Tormey, D.C., and Geelhoed, G.W.: Current Concepts in the Management of Breast Cancer. Contemporary Surgery 5: 9-20, 1974.

14. Broder, L., and Tormey, D.C.: Combination Chemotherapy of Carcinoma of the Breast: A Review. Cancer Treatment Reviews, in press, 1974.

15. Waalkes, T.P., Gehrke, C.W., Zumwalt, R.W., Chang, S.Y., Lakings, D.B., Tormey, D.C., and Ahmann, D.L.: The Urinary Excretion of Nucleosides of Transfer Ribonucleic Acid by Patients with Advanced Cancer. Accepted for publication in Cancer, 1974.

16. Tormey, D.C., Ellison, R.R., and Hossfeld, D.K.: Concurrent Monoclonal IgM and IgA Proteins in Lymphocytic Lymphoma. Accepted for publication in Cancer, 1974.

17. Tormey, D.C., and Ingle, J.N.: Diagnostic Considerations in Breast Cancer. Accepted for publication in An Atlas of Nuclear Medical Procedures in Breast Carcinoma (eds. Jones, E., and Johnston, G.), 1975.

18. Tormey, D.C., Waalkes, T.P., Ahmann, D., Gehrke, C.W., Zumwatt, R.W., Snyder, J., and Hansen, H.: Biologic Markers in Breast Carcinoma. I. Incidence of Abnormalities of CEA, HCC, Three Polyamines, and Three Minor Nucleosides. Accepted Cancer, 12/74.

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22. Richman, S.D., Brodey, P.A., Frankel, R.S., de Moss, E.V., Tormey, D.C., Johnston, G.S.: Breast Scintigraphy with ^{99m}Tc-Pertechnetate and ^{67}Ga-Citrate. J. of Nuclear Medicine, accepted for publication, 1975.

23. Tormey, D.C.: Combined Chemotherapy and Surgery in Breast Cancer: A Review. Cancer, accepted for publication, 1975.

24. Tormey, D.C.: Evaluation of Tumor Cell Markers as Indicators of Residual Disease and Response to Therapy in Report to the Profession - Breast Cancer Sept. 30, 1974 (B. Levine, E. Tarwater, E.P. Vollmer eds.) Pub by Breast Cancer Program Coordinating Branch, NCI, Bethesda, MD, 1975, pps 191-199.

25. Hossfeld, D.K., Tormey, D., Ellison, R.R.: Ph' - Positive Megakaryoblastic Leukemia, Cancer, accepted for publication, 1974.

Project No. Z01 CM 06701-03 M
1. Medicine Branch
2. Medical Breast Cancer Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 to June 30, 1975

Project Title: Factors Controlling Lymphocyte Reactivity _in Vitro_

Previous Serial Number: NCI-06701

Principle Investigator: Douglass C. Tormey, M.D., Ph.D.

Other Investigators: Lynda Chiodetti and James Neifeld, M.D.

Cooperating Units: None

Man Years:

Total: 0.8
Professional: 0.1
Other: 0.7

Project Description: 1XX

Objective:

Description, isolation and identification of molecules modulating lymphocyte replication _in vitro_.

Methods Employed:

A variety of chromatographic, precipitation, centrifugation, immunologic, and other techniques are utilized in the fractionation of growth modulating principles. Various tissue culture assays are used to assess the effects of these fractions.

Major Findings:

A factor released into the medium during the first few hours of lymphocyte culture in the absence of a mitogen has been shown to be subsequently required for the expression of the action of a mitogen upon lymphocytes. This fraction has been found to be different from transferrin. It appears to be separable into 2 or 3 entities comprising at least one inhibitor and one or two growth promoting factors. Preliminary isolation of this material as well as physiologic studies of its release and its cellular requirements are underway. The effect of various major steroids on lymphocyte growth are being defined.

Significance to Biomedical Research and Program of the Institute:

This project has broad applicability to problems of altered cell growth, especially cancer and the immunologic system. The elucidation of molecular factors which physiologically control the differentiation of cells has direct potential application to therapy.

Proposed Course:

Continued work on the fractionation of these factors is in progress, as well as physiologic studies concerning their cellular interaction; the mechanism of action of various steroids in lymphocyte growth is also under investigation.

Keyword Descriptors:

lymphocytes
cell culture
immunology
mitogens
steroids

Project No. Z01 CM 06702-01 M
1. Medicine Branch
2. Medical Breast Cancer Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Hormone Dependence of Human Breast Cancer

Previous Serial Number: New Project

Principal Investigator: Marc E. Lippman, M.D.

Other Investigators: Karen Huff, B.S., Gail Bolan, B.A., Walter M. Griffin, M.S., Douglass C. Tormey, M.D., Ph.D.

Cooperating Units: E. Brad Thompson, M.D., Lab of Biochemistry, NCI, Barbara Vonderhaar, Ph.D., Lab of Biochemistry and Metabolism, NIAMDD, Dr. John Minna, Lab of Biochemical Genetics, NHLI, Richard Knazek, M.D., Lab of Pathophysiology, NCI, Patrick Walsh, M.D., Dept. of Urology, Johns Hopkins Hospital, Baltimore, Maryland

Man Years:

Total: 4.8
Professional: 1.8
Others: 3.0

Project Description:

We are investigating the mechanisms whereby steroid hormones exert their trophic or inhibitory effects on malignant tissue. These studies are performed in a variety of systems as briefly summarized below:

A. Studies of steroid hormone receptors - The first step in steroid hormone action is an obligate binding of steroid to specific cytoplasmic receptors. We are studying human breast cancer to determine the prevalency of receptors for all classes of steroid hormones, their degree of occupancy by endogenous steroid, their intracellular localization and the correlations of all of these binding characteristics with various clinical parameters most notably the response to hormonal therapy. We are performing parallel studies on human prostate using new techniques developed in our lab. We will also be examining breast and prostatic tumor samples for growth hormone and prolactin membrane receptors.

B. Studies of hormone dependence *in vitro* - We have established and/or characterized numerous lines of human breast cancer, several of which exhibit marked dependence of various hormones including estrogens, androgens insulin or growth hormone. Stimulation of macromolecular synthesis or cell division by physiologic hormone concentrations is demonstrable as well as the induction of the specific breast marker protein α lactalbumin. Anti-estrogens strongly inhibit some of these cell lines. Hormone independent mutant cell lines are currently being prepared for analysis. These cells are also being grown on an *in vitro* capillary support system which will allow further characterization of hormone dependence.

C. Studies of control by hormones of viral expression in human breast cancer in tissue culture - We have detected particulate reverse transcriptase activity in the supernatant media of some human breast cancer cell lines. We are studying the characteristics of this activity and its regulation by hormones.

D. Studies of hormone dependence *in vivo* - We have evidence that manipulation of the hormonal milieu may lead to synchronization of a hormonally dependent cell population. Synchronization of a malignant cell population *in vivo* might permit more efficacious use of cell cycle specific chemotherapeutic agents. We are studying the timing of various estrogen methotrexate combinations in the treatment of DMBA induced rat mammary carcinoma to discover if more effective antitumor effects can be achieved by such rational combinations of hormonal and cytotoxic therapy.

Keyword Descriptors:

steroids (mechanism of action)
hormone receptors
breast cancer
prostatic cancer
prolactin
growth hormone
insulin
androgens
estrogens
α lactalbumin
antiestrogens
methotrexate
DMBA

Publications:

1. Thompson, E.B., Lippman, M.E. and Anderson, C.V. A glucocorticoid responsive hepatoma grown in defined medium. J. Cell Physiol (in press).

2. Lippman, M.E., Perry, S. and Thompson, E.B. Glucocorticoid binding proteins in myeloblasts of acute myelogenous leukemia. Am. J. Med. (in press).

3. Thompson, E.B. and Lippman, M.E. Expression of Liver - Specific and other differentiated functions in Hybrids between cultured Hepatoma Cells and L Cells _in_ Gene Expression and Carcinogenesis in Cultured Liver Cells, ed by L. Gerscherson and E.B. Thompson, Academic Press, New York, 1975 (in press).

4. Lippman, M.E. Glucocorticoid Receptors _in_ Isolation of Hormone Receptors ed by Gerald S. Lesey Marcel Dekker, New York, 1975 (in press).

5. Lippman, M.E. Hormone dependent human Breast Cancer in long term tissue culture _in_ Hormones and Cancer, ed by K.K. Charyulu in Symposia Specialists Miami, 1975 (in press).

Project No. Z01 CM 03422-02 PO
1. Pediatric Oncology Branch
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Study and Treatment of Childhood Cancer

Previous Serial Number: NCI-3422

Principal Investigator: John L. Ziegler, M.D.

Other Investigators: Robert G. Graw, Jr., M.D., Geoffrey P. Herzig, M.D., Brigid G. Leventhal, M.D., Arthur S. Levine, M.D., Ian T. Magrath, M.D., David G. Poplack, M.D., Charles L. M. Olweny, M.D., and Ann Bayley, M.D.

Cooperating Units: Pathologic Anatomy Branch, NCI; Immunology Branch, NCI; Surgery Branch, NCI; Radiation Branch, NCI; Medicine Branch, NCI; Makerere University, Kampala, Uganda; University of Zambia, Lusaka, Zambia

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description:

The Pediatric Oncology Branch is a clinical investigative unit involved in the treatment and study of neoplasia in the pediatric and adolescent age groups. Treatment approaches are multidisciplinary and center on phase II, III and IV clinical trials in selected childhood tumors. The major investigative aspects emphasize: (1) intensive chemotherapy with optimal hematologic and microbiologic supportive care; (2) exploration of tumor immunology as it relates specifically to immunotherapy; and (3) the prevention, recognition, and treatment of opportunistic infections in the compromised host. More basic research is further addressed to viral oncogenesis using the techniques of molecular biology applied to the study of SV40-adenovirus and Epstein-Barr virus. Considerable attention is also focused on aspects of the doctor-patient relationship in severe illness, and to the psycho-social effects of cancer on siblings, family and community. A broadly based oncology fellowship training program is integrated into the service through weekly formal lectures, diagnostic conferences, rounds and seminars.

exploratory methods of patient support. Once tumor burden has been substantially reduced, the notion of immunological control of "minimal residual disease" becomes an attractive approach, and is under considerable study. Adjuvant immunotherapy may offer the cancer patient clinical benefit by virtue of its potential effects on: (1) non-specific immunity; (2) tumor-associated immunity; (3) tolerance to chemotherapy; (4) resistance to infection; (5) ease of remission reinduction; and (6) prophylactic effect against viral oncogenesis. Continued study of tumor immunology and immunotherapy remains a major commitment of our research effort.

Keyword Descriptors: Phase II, III and IV clinical trials, intensive chemotherapy, supportive care, tumor immunology, immunotherapy, opportunistic infections, viral oncogenesis, doctor-patient relationship, psycho-social effects of cancer, oncology fellowship training program.

Honors and Awards: John L. Ziegler, M.D.

Appointments:

1. Member, American Society of Clinical Investigation
2. Member, Scientific Advisory Committee, American Cancer Society
3. Member, Medical Board, Clinical Center, NIH

Invited Lectures: 12

Publications:

1. Ziegler, J. L., Magrath, I. T., Nkrumah, F. A., Perkins, I. V. and Simon, R. E.: Evaluation of 1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea (CCNU) for the prevention of CNS involvement in Burkitt's lymphoma. Cancer Chemother. Rep. (in press).

2. Morrow, R. H., Jr., Levine, P. H., Ziegler, J. L., Berard, C.: What is Burkitt's lymphoma? Lancet II: 1268, 1974.

3. Olweny, L. M., Mbidde, E. K., Nkwocha, J., Magrath, I., Ziegler, J.L.: Chemotherapy of Hodgkin's disease. Lancet II: 1397, 1974.

4. Perlin, E., Nielsen, P., Karp, D., Watne, A., Ziegler, J. L.: Therapy of Burkitt's lymphoma in the central nervous system. Ann. of Intern. Med. 81: 408-409, 1974.

5. Taylor, J. F. and Ziegler, J. L.: Cellular immunity in Kaposi's sarcoma. Brit. J. Cancer 30: 312-318, 1974.

6. Ziegler, J. L. and Leventhal, B. G.: Treatment of acute leukemia. In Peschle (Ed.): Aplastic Anemia and Acute Leukemia (in press).

The specific objectives of the salient clinical and related laboratory studies, the highlights of progress in each area, and the future plans will be summarized in this report. The Branch consists of three Sections: Chemoimmunotherapy, Experimental Hematology, and Infectious Diseases, and individual section reports will elaborate further on progress and plans as each topic relates to the activities of that section.

Objective:
To observe the effect of intermittent immunotherapy (BCG and allogeneic tumor cell vaccine) versus methotrexate on remission duration and relapse in maintenance therapy of acute lymphocyte leukemia.
Progress:
82 patients have entered the protocol study; a complete remission rate of 95% was achieved. Remissions after CNS prophylaxis were terminated by meningeal leukemia in only four patients, and the median remission duration is in excess of 36 months. Chemoimmunotherapy was no more beneficial than chemotherapy alone in remission. Considerable new data regarding the serial immune status (immunocompetence and tumor-associated reactivity) of these patients are under analysis.
Plan:
Prognostic discriminants (WBC, ease of remission induction, CNS leukemia) have characterized "standard" and "poor" risk groups, and may also correlate with certain immunological features of leukemia blasts. Utilizing these features a new protocol has now been devised which will deliver more intensive therapy to high risk patients and explore the role of immune adjuvants in improving host resistance and tolerance to chemotherapy.

Objective:
To optimize host tolerance of high dose chemotherapy using bone marrow allografting or autografting in a canine preclinical model and in man.
Progress:
During the past year an intensive chemotherapy regimen (cyclophosphamide, cytosine arabinoside, thioguanine, BCNU) was administered to 16 patients with drug-resistant leukemia or lymphoma in relapse. Significant tumor regression was achieved in all evaluable patients, although early (occasionally fatal) cardiac toxicity was a major problem. Evidence of successful marrow engraftment was observed in all evaluable patients. Several prolonged remissions were achieved, and results with marrow autografting appear particularly promising.
Plan:
Modification of cyclophosphamide dosage appears to obviate fatal cardiac toxicity, and further efforts at modifying graft versus host disease (marrow allograft) are underway. Studies of marrow autografting will be attempted in childhood solid tumors (sarcoma and lymphoma).

Objective:
Ascertain the utility of granulocyte transfusion (comparing two collection techniques) in patients with leukopenia and sepsis in a controlled randomized trial.
Progress:
Preliminary results in 26 randomized patients indicate efficacy of WBC transfusion in patients who fail to recover marrow function during the study period. Leukopak WBC collections are associated with more transfusion reactions than IBM cell-separator collections, and demonstrate increased stickiness necessitating four-fold greater increments for equivalent clinical efficacy.
Plan:
The trial will continue until data indicate a definitive trend. Trials of "prophylactic" WBC transfusion in prevention of sepsis will also be undertaken in patients receiving high dose chemotherapy. Study of leukocyte physiology and function related to collection techniques will continue.

Objective:
Combined modality treatment of American Burkitt's lymphoma and a randomized trial of immunotherapy.
Progress:
Two of 14 patients who have entered this study died during induction and the remainder (all with advanced disease) achieved complete remissions. Five patients remain in their first remission 10+ - 26+ months, and seven have relapsed (five dead, two living). Immunotherapy with BCG and Raji cells achieved high titres of cytotoxic antibody in all three randomized patients, one of whom relapsed; two control subjects developed no antibody and remain in sustained remission.
Plan: A new protocol is being prepared which will intensify chemotherapy following remission induction in patients with advanced stages; experimental bone marrow autografting will be assessed as a supportive care measure in relapsing patients. Passive immunotherapy using plasma from patients with anti-Raji cytotoxic antibody (vs. controls) will be examined in patients who relapse with readily measurable tumor (i.e. bone marrow).

Objective:
To improve management of meningeal leukemia through the use of the Ommaya reservoir and measured methotrexate (MTX) levels in the CNS in a randomized trial.
Progress:
To date 14 patients have been studied; 7 patients received "CXT" therapy using MTX injections q12h x 3d adjusting doses to achieve levels of 5×10^{-7}M; 7 patients received conventional MTX via the reservoir. The median duration of CSF remissions is similar (192 and 154 days respectively) and the CXT regimen appears to be better tolerated.
Plan:
In view of increasing reports of CNS toxicity from MTX, continued efforts to develop safe and effective intrathecal MTX therapy are mandatory. The

present trial has been modified slightly based on preliminary results, and will continue.

Objective:
To monitor the incompetence of children with leukemia and solid tumors to assess the effect of tumor burden, chemotherapy, and immunotherapy.
Progress:
This study has been underway for ten months, and has been integrated into the routine patient evaluation. Preliminary data indicate that various chemotherapy regimens differ in immuno-suppressive toxicity, and that tumor burden exerts an independent non-specific and tumor-associated immunological impairment. The kinetics of immune recovery following treatment can be documented. Such data will assist in the design of appropriate immunotherapeutic intervention.
Plan:
This study will continue, and will develop pertinent assays of macrophage and lymphocyte function in a search for relevant clinical correlates.

Objective:
To detect, characterize and purify leukemia-associated antigens for ultimate use in immunotherapy of acute leukemia.
Progress:
Collaborative research has established a variety of assays of leukemia associated antigens. Leukemia blast cells share a dedifferentiation antigen with the Raji cell line, as well as functional surface characteristics. As in Burkitt's lymphoma, high titres of cytotoxic antiserum can be elicited in patients with ALL using a Raji cell vaccine, and a current trial seeks to optimize the vaccine schedule using BCG "priming." Further characterization of leukemia cells using mixed leukocyte reactivity (MLR) and E rosetting techniques may distinguish at least two major subgroups: a T-cell leukemia non-reactive in MLR and associated with high WBC and mediastinal tumors; and a T_0-cell leukemia reactive in MLR. Clinical correlates of these subgroups are under examination to detect possible prognostic discrimination.
Plan:
This promising research will continue along the same lines, since new cellular characteristics are evolving which will supplement morphological and clinical studies related to natural history, treatment, and prognosis of leukemia.

Objective:
To study factors related to optimal platelet transfusion responses.
Progress:
Since the discovery of platelet compatibility related to the HL-A system, a computerized donor-recipient matching program has been developed utilizing a pool of 9,500 HL-A typed donors. The "NIH Plateletpheresis Center" has been

established to improve our capability to provide matched platelets in the NIH, the community and nationally.

Plan:

Techniques to detect other aspects of platelet cross-matching such as platelet aggregation, lymphocyte dependent antibody, serotonin release assays and the two-step platelet antibody-macrophage assay, will be pursued. Further development of the National Platelet Donor Pool will continue.

Objective:

To develop postgraduate-specialty education programs for nurses, medical students and oncology fellows.

Progress:

During the past year a highly successful five-month Pediatric Oncology Nurse Training Course was developed; six nurse graduates completed the course and have been integrated into the Clinical Service. Regularly scheduled rounds, conferences and seminars in the Branch have addressed major topics in clinical and investigative oncology, aspects of the doctor-patient relationship, and psycho-social factors in childhood neoplasia.

Plans:

Clinical Associates have reacted very favorably to the Branch commitment to education. These programs will continue, and supplementary hematology elective sessions to satisfy Pediatric Hematology-Oncology Board requirements will be explored.

Objective:

To develop combined modality treatment programs in childhood solid tumors.

Progress:

Ten patients with rhabdomyosarcoma have entered an Intergroup Cooperative Protocol, and excellent clinical responses have been achieved. Of interest is a collaborative study with the Pathology Branch and NHLI, in which antisera to myosin protein can be used to distinguish fibroblastic, myoblastic or embryonal myosin by an immunofluorescent technique. This will aid greatly in histological differentiation of sarcoma types, and may provide a basis for a serum tumor marker.

Four patients with osteosarcoma are under study: two with metastatic tumors are treated with cyclophosphamide/adriamycin alternating with high dose methotrexate, and two are receiving post-amputation adjuvant chemotherapy in a collaborative study with the Medicine Branch. Several patients with miscellaneous tumors (teratoma secreting alpha-feto protein, chemodectoma metastatic to lung) are under individual study.

Plan:

These pilot studies have indicated that successful collaborative combined modality trials in pediatric tumors are feasible in the NIH Clinical Center, and have provided important baseline data on the natural history and responsiveness of these tumors. New collaborative protocols for adjuvant therapy of childhood sarcomas and childhood non-Hodgkin's lymphomas are directed at questions of early intensive chemotherapy with optimal supportive care, and aggressive treatment of relapse.

Objective:

To study opportunistic infections in the compromised host with cancer, and to develop menas of prophylaxis in patients at high risk.

Progress:

A controlled evaluation of the limulus assay for the detection of endotoxemia revealed limited clinical usefulness with poor concordance between positive tests and positive blood cultures and numerous false negative results. *Pseudomonas Aeruginosa* vaccine was given to 34 patients, with 32 showing substantial antibody responses. A clearly prophylactic effect, however, could not be demonstrated (5 subjects developed *Pseudomonas* bacteremia).

Plan:

In May, 1975, a six-bed laminar flow room/intensive care unit will be opened on the 2B corridor. Initial trials are directed to the treatment of patients with acute leukemia with high-dose intensive chemotherapy (followed in some by bone marrow transplantation) in or out of the laminar air flow rooms in a randomized study. Frequency of infection, drug tolerance, treatment response, and survival will be observed. The hypothesis to be tested is that laminar air flow room protection will improve tolerance to intensive therapy and allow the latter to ablate tumor.

Objective:

To study molecular mechanisms of viral oncogenesis *in vitro* using the SV-40 adenovirus hybrid and Epstein-Barr virus systems.

Progress:

A major finding has been the observation of tandem repetition of SV-40 DNA in cells infected with defective hybrid viruses (Ad^{++} HEY and Ad2^{++} LEY), implying that differences in rate of excision of SV-40 DNA from hybrid genomes may be a determinant for lytic infection or transformation. In seeking to correlate AKR leukemia viral DNA sequences with leukemogenesis, it was found that frequency of leukemia in different strains of mice correlated with the completeness and number of copies of the murine leukemia virus genome in cellular DNA. Studies in Epstein-Barr virus have succeeded in improving the yield of purified virus using various cultural modifications.

Plan:

Continued studies of viral oncogenesis will explore genomic determinants of neoplastic transformation in DNA and RNA oncogenic viruses or hybrids, with the aim of further understanding molecular control of cell transformation.

Summary and Relevance to NCI Program

Major progress have been achieved toward attaining the objectives outlined above. In particular, improved knowledge of hematologic and microbiologic supportive care combined with clear evidence of dose-response phenomena in the chemical control of neoplasia have led to the general hypothesis that better treatment results can be achieved through higher doses of drug in the tolerant host. Although some obstacles exist in this approach (e.g. extramedullary toxicity, sanctuary tumor, primary drug resistance) it is our intention to explore the use of intensive chemotherapy utilizing known and

7. Bodmer, J. G., Bodmer, W. F., Ziegler, J. and Magrath, I. T.: HL-A and Burkitt's tumor. A study in Uganda. *Tissue Antigens* (in press).

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9. Ziegler, J. L.: The design and planning of controlled clinical therapeutic trials. In Bucalosi, *et al*. (Eds.): *Ilinfomi Maligni*. Milan, Casa Editrice Ambrosiana, 1974, pp. 181-182.

10. Schein, P. S., O'Connell, M. J., Blom, J., Hubbard, S., Magrath, I. T., Bergevin, P., Wiernik, P. H., Ziegler, J. L. and DeVita, V. T.: Clinical antitumor activity and toxicity of streptozotocin (NSC-85998). *Cancer* 34: 993-1000, 1974.

11. Ziegler, J. L.: Progress in the lymphomas. In Proceedings of the International Congress of Pediatrics, Buenos Aires, 1974.

12. Primack, A., Vogel, C. L., Kyalwazi, S. K., Ziegler, J. L. and Simon, R.: A staging system for hepatocellular carcinoma: Prognostic factors. *Cancer* (in press).

13. Ziegler, J. L.: Management of Burkitt's lymphoma. *Pediat. Ann*. 4: 60-71, 1975.

14. Ziegler, J. L.: Management of Burkitt's lymphoma. In Pochedly (Ed.): *Management of Cancer in Children*. Acton, Publishing Sciences Group, 1975, pp. 187-202.

Appendix A

Contract Number: N01-CM-71343-07 PO

Contractor: Makerere University Medical School (Uganda Cancer Institute),
Kampala, Uganda

Principal Investigators: Professor S. K. Kyalwazi and Dr. Charles L. M. Olweny

Budget: $251,681

Man Years:		
	(Professional)	4
	(Other)	7

Objective:

To study the treatment of Burkitt's lymphoma, hepatocellular carcinoma, and Kaposi's sarcoma in controlled clinical trials.

Progress:

The Uganda Cancer Institute is a self-contained 40-bed clinical research hospital where selected patients are admitted for protocol study and followup. Patients with Burkitt's lymphoma receive at random either cyclophosphamide (1000 mg/m^2) or a cyclophosphamide-oncovin-methotrexate (COM) combination for two courses, followed by further randomization to craniospinal radiation (CSIR) or no CSIR in patients without meningeal involvement on presentation. Results to date indicate:

Cyclophosphamide (17)		COM (18)	
CR	PR/NR	CR	PR/NR
12 (66%)	5	16 (88%)	2

CSIR		No CSIR	
No.	No. Relapse	No.	No. CNS Relapse
7	2	7	3

Thus COM therapy is superior to cyclophosphamide in remission induction, and CSIR appears not to offer protection against the development of meningeal involvement.

Patients with hepatocellular carcinoma are treated with systemic adriamycin (preceded in some by hepatic artery ligation) or a combination of adriamycin and dichloromethotrexate. Current results reveal:

Adriamycin			Adriamycin-DCM		
CR	PR	NR	CR	PR	NR
4	18	14	0	1	7

Responses were generally brief, but survival was prolonged by several months in responding patients. Large doses of adriamycin (70 mg/m^2) were required to achieve response, and the cardiotoxic dose limit prevented further treatment. Current efforts are directed at intra-hepatic arterial adriamycin as a means of increasing tumor exposure to drug and lowering plasma levels. Collaborative pharmacologic studies of tissue and plasma drug levels are underway at the BCRC.

A trial in Kaposi's sarcoma has been recently completed (Olweny et al., 1974) showing the superiority of a three drug combination containing actinomycin D, vincristine and imidazole carboximide (94% CR) over actinomycin and vincristine (55% CR). Further studies of maintenance chemotherapy, immunotherapy or no maintenance therapy are underway.

Numerous collaborative studies of viral etiology, tumor markers, EBV serology and tissue studies are supported through this contract. In addition other tumor types (Hodgkin's disease, histiocytic lymphoma, Wilm's tumor, neuroblastoma, esophageal cancer) are studied in ancillary protocols.

Plan:

The work scope of this contract is to be reduced with emphasis on patient followup from previous protocols, chemotherapy of hepatocellular carcinoma, and support for collaborative tissue and serum studies.

Bibliography:

1. Olweny et al.: Treatment of Kaposi's sarcoma by combination of Actinomycin-D, Vincristine and Imidazole Carboxamide (NSC 45388): Results of a randomized clinical trial. Int. J. Cancer 14: 649-656, 1974.

2. Olweny et al.: Chemotherapy of Hodgkin's disease. Lancet II: 1397, 1974.

3. Olweny et al.: Treatment of Hepatocellular carcinoma with Adriamycin: Preliminary communication. Cancer (in press).

4. Magrath, I. T., Semaware, C. and Nkwocha, J.: Causes of death in patients with Burkitt's lymphoma. The role of supportive care in overall management. *E. Afr. Med. J.* 51: 623-632, 1974.

5. Magrath, I. T., Henle, W., Owor, R. and Olweny, C.: Antibodies to Epstein-Barr virus antigens before and after the development of Burkitt's lymphoma in a patient treated for Hodgkin's disease. *New Eng. Med. J.* (in press).

6. Magrath, I. T. and Henle, W.: Changes in antibodies to Epstein-Barr virus associated antigens with the development of Burkitt's lymphoma. Proc. of the Second International Symposium on Oncogenesis and Herpes Viruses (in press).

Appendix B

Contract Number: N01-CM-43764-01 PO

Contractor: Emory University, Atlanta, Georgia

Sub-Contractor: University of Zambia, Lusaka, Zambia

Principal Investigators: Dr. Charles L. Vogel and Dr. Anne Bayley

Budget: $17,373

Man Years: 1 1/2

Objective:

To undertake a phase II study of adriamycin in hepatocellular carcinoma in Zambia, and to study biochemical tumor markers as indicators of tumor response.

Progress:

During the first six months of the study (September 1974-March 1975) 13 patients have entered the trial of whom 9 are evaluable. One objective tumor response was noted, and three patients showed partial subjective improvement. Doses of adriamycin were substantially reduced in virtually all patients because of hyperbilirubinemia, and it was felt by the investigators that the relatively poor responses are attributable in part to the reduced dosage.

Plan:

The trial will continue to accrue patients, but adriamycin doses will be increased. A site visit is planned in June 1975 to ascertain feasibility of contract continuation and/or expansion.

Bibliography: None

Project No. Z01 CM 06810-11 PO
1. Chemoimmunotherapy Section
2. Pediatric Oncology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Chemoimmunotherapy

Previous Serial Number: NCI-6810

Prinicpal Investigator: Brigid G. Leventhal, M.D.

Other Investigators: David G. Poplack, M.D., Lucio Crino, M.D., Dean L. Mann, M.D., John L. Ziegler, M.D. and W.A. Bleyer, M.D.

Cooperating Units: Immunology Branch, NCI
Clinical Center, NIH
Department of Pediatrics, University of Washington, Seattle

Man Years:

Total:	14 1/2
Professional:	4 (excluding 1st year Clinical Associates)
Other:	10 1/2

Project Description:

Objectives:

1) To detect, characterize and purify tumor antigens in acute leukemia.

2) To assess the effect of chemotherapy and immunotherapy on the immune response to tumor and other antigens.

3) To devise effective therapeutic regimens which allow maximal tumor cell kill with drugs and with immunotherapy while leaving intact effective elements of the immune response.

4) To assess the pharmacology of selected agents such as methotrexate (MTX) in blood and CSF.

5) To improve methods for the safety and efficacy of the delivery of health care with emphasis on the involvement of nonphysician health professionals.

6) To study the long-term effects of therapy, particularly physical and psychological development.

Methods Employed:

A. Educational:

1. Pediatric Oncology Nurse: For the first time a 5-month course has been given to nurses in an attempt to develop a "Pediatric Oncology Nurse." This course was supervised in collaboration with Ms. June McCalla, M.A., R.N., and involved the development of many new teaching techniques and concepts. On 3/14/75 six graduates of the program assumed their expanded role in the Clinical Center. It is our plan that this specialized education will lead to more effective patient care. We feel that subspecialization within nursing is a desirable and inevitable prospect for the future.

2. Pediatric Grand Rounds: These are held monthly in collaboration with Dr. Joseph Schulman, NICHD, with speakers invited from within NIH and off campus in an attempt to develop a sense of consciousness of an NIH pediatric community and to broaden the scope of our pediatric training.

Major Findings:

A. Leukemia antigens and the nature of the cell of origin of leukemia:

1. It has been recently reported that the human T cell does not stimulate allogeneic lymphocytes in mixed leukocyte culture. We have analyzed the capacity of the blast cells of our patients to stimulate in MLC and have discovered that cells from 11 patients did not stimulate while 20 patients had cells which did stimulate. The group which did not stimulate could be further divided into five whose cells clearly did form E rosettes and two whose cells did not form E rosettes. In the group which did stimulate all patients tested (4) did not form E rosettes. None of the patients' blasts formed EAC' rosettes. We speculate that ALL can be divided into at least two categories. The first group of patients, those whose cells do not stimulate in MLC, probably have a disease which represents proliferation of more mature T cells: those which had been under some thymic influence. The other group of patients probably have a disease which represents proliferation of the lymphoid stem cell in the bone marrow. It appears that the latter group are probably those with a better prognosis.

B. Measurement of the immune response:

1. A broad immune evaluation protocol (NCI 74-9) has been developed which includes the study of both cellular and humoral lymphocyte responses in patients as well as development of new assays for macrophage function.

2. The macrophage assays include macrophage mediated antibody cytotoxicity which has been studied and found to be defective in patients with Wiskott-Aldrich syndrome and possibly in patients after bone marrow

transplantation; the uptake of colloidal Au^{198} which, when studied in guinea pigs was found to be increased after the administration of BCG; and macrophage monocyte chemotaxis. The ability to perform these studies will add a new dimension to our understanding of possible antitumor immune mechanisms in vitro.

C. Therapeutic regimens in Acute Lymphatic Leukemia (ALL) (Protocol 72-1):

1. Previously untreated children with ALL were treated on a protocol designed to compare the effectiveness of maintenance chemotherapy (CT) alone vs. CT + immunotherapy (IMT). Patients received induction therapy with POMP followed by cranial irradiation with 2400R plus i.t. methotrexate (MTX) or cytosine arabinoside (AraC). There was additional consolidation in half the patients with two 5-day courses of AraC and 6 thioguanine.

Maintenance randomization was to either CT alone (4 months of intermittent prednisone plus vincristine, weekly MTX and daily 6MP alternating with 2 months of MTX alone) or to CT + IMT (4 months P, VCR, MTX and 6MP alternating with 2 months of IMT) for 34 months. IMT was fresh Pasteur Institute BCG given by multiple puncture plus intradermal irradiated allogeneic leukemia cells from a single donor given in 4 weekly doses for the first month of the 2-month period. 49 patients were randomized for maintenance therapy. There was no significant difference in relapse rate (9/30 on CT and 7/19 on CT + IMT). 59% of all patients are in complete remission at 30 months.

2. Immunologic response in both regimens was monitored with skin tests and in vitro lymphocyte stimulation. There was progressive immunosuppression with continuous chemotherapy over the duration of treatment. Although vigorous specific reactivity to tuberculin occurred, there was no nonspecific stimulation of immune reactivity with IMT. We conclude that BCG and allogeneic cells did not increase the effectiveness of CT alone in ALL.

D. Protocol 74-7:

1. The COAP + RAJI protocol was discussed in last year's annual report. Patients randomized in that protocol to CT + IMT made antibody which was cytotoxic to RAJI and leukemia cells in vitro, but contained lymphocyte dependent antibody to RAJI only and not to leukemia cells. There was a temporary increase in in vitro MLC response to RAJI cells as well. This immune response to immunization was encouraging, but no effect on the clinical course of the patients' disease was seen.

2. We are now engaged in a protocol for patients with acute leukemia in relapse using POMP chemotherapy and immunotherapy with RAJI ± BCG, varying schedules in sequential patients. We hope with this to discern an

immunotherapy schedule which will allow the broadest possible immune response to tumor antigen in man. Once this has been identified, further therapeutic trials are planned.

E. Protocol 75-1:

1. A great deal has been learned about prognostic factors in ALL. Morphologic criteria (size, etc.) were analyzed and found not to be helpful. However, certain other features such as meningeal leukemia at first LP (5/5 relapsed) failure to achieve CR with four courses of therapy (7/7 relapsed) and initial white count > 50,000 per mm^3 without any such factors (5/10 relapsed) led to a poor prognosis in 72-1. Of patients with those factors, 80% are in remission at three years.

2. It is planned that patients will be divided into good and poor prognosis groups early in the course of their disease. The good prognosis group will receive conventional therapy with vincristine, prednisone, 6 mercaptopurine and methotrexate. All patients will receive cranial irradiation and methotrexate. Poor prognosis patients will receive in addition intensified chemotherapy with asparaginase and high dose cytoxan during induction, and six months after remission is achieved a pulse of AraC, 7 days and daunorubicin, 3 days. Both groups of patients will be randomized to receive or not receive MER. This agent, a methanol extractable residue of BCG, has been selected for immunotherapy because it has had demonstrated stimulatory effects on the immune response in man receiving concurrent chemotherapy, and in animal systems where it is effective, unlike BCG, it can be given at sites distant to the tumor.

F. Pharmacologic studies:

1. The study of the effect of repeated small doses of MTX given intrathecally via Ommaya reservoir (C x T therapy) compared with higher pulse doses continues, and additional patients are being entered into the protocol from the Department of Pediatrics in Seattle in collaboration with Dr. W.A. Bleyer.

G. Safety and efficacy of therapy:

1. The development of pediatric oncology nurses has been discussed under methods. Ancillary studies which involve active participation of the nurses include the assessment of the risk of family members of patients who are chronic carriers of HBsAg contracting hepatitis. It was discovered that 4/25 mothers of such patients developed icteric hepatitis and 4/40 siblings sero-converted indicating clinical infection in a group of 84 relatives of 29 patient carriers of HBsAg. No family members of non-carrier patients developed hepatitis during the same period of time. In the families where infection occurred oral contact such as shared food and kissing were common and HBsAg was identified in

the saliva of several patients. It is concluded that oral contact is a significant mode of spread of hepatitis and that families should be counseled appropriately when the patient is discovered to have circulating HBsAg.

H. Long-term effects of the therapy:

1. The effects of the disease on patients and their families are studied during a regular meeting with parents conducted with the social worker, Ms. J. Hagopian. A similar conference with a consultant psychiatrist, Dr. Stephen Hersh, is held weekly for staff.

2. Studies of the effect of chemotherapy on growth and development of sexual maturation and function in girls has been conducted with Drs. Siris and Vaitukaitis and in boys with Dr. Richard Sherrins. It appears that the majority of patients have relatively normal maturation, despite the duration of chemotherapy, as long as they have not received alkylating agents.

3. Assessment of long-term toxicity, particularly to the nervous system, is underway. Three disturbing cases of severe visual toxicity in patients with ALL (two with total blindness) have been seen during the past year. In addition a number of patients are being seen with progressive deterioration of mental function despite remission disease status. Studies to help understand the nature of this process and to assess its frequency are underway.

Significance to biomedical research and the program of the Institute:

A. Treatment of leukemia:

1. Significant progress has been made in delineating the cell of origin of acute leukemia. It appears that there are at least two groups of patients, one with a "null" cell tumor which does stimulate in MLC and one with a "T" cell tumor which does not stimulate in MLC. These two types of disease have a different prognosis. Therapeutic implications of this discovery are being examined. Our unique capability for broad studies of the immune response combined with special studies of antigens in leukemia will allow us to contribute maximally to the understanding of the anti-tumor immune response in man.

B. Quality of life for the cancer patient:

1. Treatment, to be truly effective must provide life which is of good quality as well as of increased quantity. Ancillary studies are being instituted on problems such as growth, development of sexual function, effect of therapy on psychological and mental development and hazards both physical (such as hepatitis) and psychological for the families

of the patients. Methods are being developed for maximal use of non-physician professionals in these areas.

C. Proposed course:

1. In our current protocols attention will be paid to prognostic factors so that patients at greatest risk will receive the most vigorous therapy early in their disease.

2. In all studies attention will be paid to a broad spectrum of possible effects of therapy on the patient. Therapeutic effects will be studied as well as long- and short-term toxic effects of therapy. New record keeping methods will be developed for assessment of immunologic effects, metabolic effects and psychological effects of therapy.

Honors and awards:

Brigid G. Leventhal, M.D.

1. Board certification in Pediatric Hematology and Oncology, 1974.

2. American Society for Clinical Investigation.

Chairmanships:

1. Chairman, ALGB Immunotherapy Subcommittee, 1974.

2. Co-chairman, Oncology Section, ASCI Meeting, 1975.

3. Co-chairman, Immunotherapy Section, ASCO Meeting, 1975.

Invited Lectures:

1. American Cancer Society Conference on Childhood Cancer, Dallas, Texas, March, 1974.

2. Conflicts in Childhood Cancer, Roswell Park Memorial Institute, Buffalo, New York, 1974.

3. Recent Developments in Childhood Cancer, Childrens' Hospital of Philadelphia and American Oncologic Hospital, Philadelphia, Pennsylvania, 1974.

4. American Cancer Society First Annual Symposium on Cancer at Disney World, Florida, February, 1975.

5. Symposium on Childhood Cancer, University of North Carolina, Chapel Hill, North Carolina, March, 1975.

Publications:

1. Leventhal, B.G. and Hersh, S.: Treatment of acute leukemia: the patient and his family. Children Today 3: 2-6, 1974.

2. Yust, I., Wunderlich, J., Mann, D.L., Rogentine, G.N. Jr., Leventhal, B.G., Yankee, R. and Graw, R.G.: Human lymphocyte dependent antibody mediated cytotoxicity and direct lymphocyte cytotoxicity against non-HLA antigens. Nature 249: 263-265, 1974.

3. Mann, D.L., Halterman, R. and Leventhal, B.G.: Acute leukemia associated antigens. Cancer 34: 1446-1451, 1974.

4. Graw, R.G., Lohrmann, H.P., Bull, M.I., Decter, J., Herzig, G.P., Bull, J.M., Leventhal, B.G., Yankee, R.A., Herzig, R.H., Krueger, G.R.F., Bleyer, W.A., Buja, M.L., McGinniss, M.H., Alter, H.J., Whang-Peng, J., Gralnick, H.R., Kirkpatrick, C.H. and Henderson, E.S.: Bone marrow transplantation following combination chemotherapy immunosuppression (B.A.C.T.) in patients with acute leukemia. Transplantation Proceedings 6: 349-354, 1974.

5. Shohet, S.B., Pitt, J., Baehner, R.L. and Poplack, D.G.: Lipid peroxidation in the killing of phagocytized pneumococci. Infection and Immunity 10: 1321-1328, 1974.

6. Fraumeni, J.F. Jr., Wertelecki, W., Blattner, W.A., Jensen, R.D. and Leventhal, B.G.: Varied manifestations of a familial lymphoproliferative disorder. Am. J. Med, (in press) 1975.

7. Leventhal, B.G.: New looks in leukemia. Cancer 35: 1015-1021, 1975.

8. Mann, D.L., Leventhal, B.G. and Halterman, R.: Human antisera detecting leukemia-associated antigens on autochthonous tumor cells. J. Natl. Cancer Inst. 54: 345-347, 1975.

9. Leventhal, B.G.: What's new and promising in leukemia and lymphoma. In Seydel, G. and Donaldson, M. (Eds.): Symposium Proceedings: Recent Developments in Childhood Cancer. New York, John Wiley and Sons (in press), 1975.

10. Leventhal, B.G., Levine, A.S., Graw, R.G., Henderson, E.S., Simon, R., and Freireich, E.J.: Long term second remission in acute leukemia. Cancer (in press).

11. Leventhal, B.G.: Immune status in acute leukemia in Proceedings of the Symposium on Conflicts in Childhood Cancer. American Assoc. for Research on Childhood Cancer (in press).

12. Mann, D.L., Halterman, R. and Leventhal, B.G.: Leukemia associated antigens detected with serums from immunized patients. Cancer Research (in press).

13. McCalla, J., Leventhal, B.G., Harpine, F. and Furguson, V.: A description of a short term continuing education program for the development of a pediatric oncology nurse. J. Cont. Educ. Nurs. (in press).

14. Pizzo, P.A., Henderson, E.S. and Leventhal, B.G.: Acute myelogenous leukemia in children: A preliminary report. J. Pediat. (in press).

Keyword Descriptors: tumor antigens, acute leukemia, chemotherapy, immunotherapy, immune response

Appendix A

Contract Number: NO1-CM-12127

Contractor: Hebrew University, Jerusalem, Israel

Principal Investigators: Dr. David Weiss and Dr. Gaby Izak

Budget: $115,434

Man Years: 6

Objective:

To investigate the therapeutic effect and the effect on the immune response of MER (methanol extraction residue of BCG) in tumor models and in patients with AML.

Progress:

To date 26 patients with AML have been entered on a randomized study who receive maintenance therapy either alone or in combination with MER. Survival and remission duration are longer, though not significantly so in the MER group than in the group receiving chemotherapy alone. Laboratory studies have shown a more vigouous 19S antibody response to primary immunization with KLH in the MER group than in the control groups. MER effects on the immune response in animals is also being investigated.

Plan:

The contract will continue to accrue patients for at least two years. Careful monitoring will be required during that time.

Bibliography:

1. Weiss, D.W., Stupp, Y., Manny, N. and Izak, G.: Treatment of acute myelocytic leukemia (AML) patients with the MER tubercle bacillus fraction. A preliminary report. Transplant. Proc. (in press), 1975.

2. Gery, I., Baer, A., Stupp, Y. and Weiss, D.W.: Further studies on the effects of a Methanol Extraction Residue fraction of tubercle bacilli on lymphoid cells and macrophages. In: Immunological Parameters of Host-Tumor Relationships, Vol. III, D.W. Weiss, eds., Academic Press, New York, 170-177, 1974; and, in Israel J. Med. Sci., 10: 984-991, 1974.

3. Ben-Efraim, S., Teitelbaum, R., Ophir, R., Kleinman, R. and Weiss, D.W.: Nonspecific modulation of immunological responsiveness in guinea pigs and mice by the MER mycobacterial fraction: Influence of conditions of MER treatment and specific immunization, and effect of MER on early stages in

the immune response. In Weiss, D.W. (ed.): Immunological Parameters of Host-Tumor Relationships. New York, Academic Press, 1974, Vol. III, pp. 158-169, 1974; and in *Israel J. Med. Sci*., 10: 972-983, 1974.

4. More, R., Yron, I., Ben-Sasson, S. and Weiss, D.W.: *In vitro* studies on cell-mediated cytotoxicity by means of a terminal labeling technique. *Cell. Immunol*., 15: 382-391, 1975.

5. Steinitz, M. and Weiss, D.W.: Studies on the physiological manifestations of cell-mediated cytotoxicity. I. Early metabolic changes in mouse plasmacytoma cells exposed *in vitro* to sensitized allogeneic splenocytes. *Cell. Immunol*., 15: 403-418, 1975.

6. Steinitz, M. and Weiss, D.W.: Studies on the physiological manifestations of cell-mediated cytotoxicity. II. Inhibition of H^3 thymidine incorporation by plasmacytoma cells exposed *in vitro* to sensitized splenocytes. *Cell. Immunol.* (in press), 1975.

7. Wainberg, M., Doljanski, F., Markson, Y. and Weiss, D.W.: Cellular immunity against Rous sarcomas of chickens. Preferential reactivity against autochthonous target cells as determined by *in vitro* immunoadherence and cytotoxicity tests. *Proc. Nat. Acad. Sci*. 71: 3565-3569, 1974.

8. Doljanski, F., Markson, Y., Wainberg, M.A. and Weiss, D.W.: Cellular immunity to Rous sarcoma (RS) - Preferential autochthonous interactions; and, application of the immunoadherence technique to mouse tumors. *Transplant. Proc*. (in press), 1975.

9. Wainberg, M., Markson, Y., Doljanski, F. and Weiss, D.W.: Reactivity of serum from Rous sarcoma-bearing chickens with autochthonous and with allogeneic tumor cells: Preferential autochthonous recognition. *Int. J. Cancer* (in press), 1975.

10. Weiss, D.W.: Immunological intervention in neoplasia. Seventh Miles International Symposium: The Role of Immunological Factors in Viral and Oncogenic Processes. The Johns Hopkins Journal, supplement no. 3. R.F. Beers, Jr., R.C. Tilghman and E.G. Basset, eds., The Johns Hopkins University Press, Baltimore, Maryland, 131-156, 1974.

11. Weiss, D.W.: Discussion on: Immunotherapy and Immunoprophylaxis of Cancer: The application of modulators of immunological responsiveness. Seventh Miles International Symposium: The Role of Immunological Factors in Viral and Oncogenic Processes. The Johns Hopkins Journal, supplement no. 3. R.F. Beers, Jr., R.C. Tilghman and E.G. Basset, eds., The Johns Hopkins University Press, Baltimore, Maryland, 157-169, 1974.

Appendix B

Contract Number: N01-CM-23701

Contractor: Hazleton Laboratories American, Inc.

Principal Investigator: Charles A. Bowles, Ph.D.

Budget: $131,817

Man Years: 3 1/2

Objective:

Performance of mixed leukocyte cultures in dogs and man.

Progress:

During the contract year the laboratory has processed 249 human and 910 canine blood specimens. Some of this data was generated on the *in vitro* response of patients on the 72-1 protocol to antigens mitogens and in MLC. Family MLC's to screen candidates with leukemia and aplastic anemia for possible bone marrow transplantation were also performed. Longitudinal studies of normal and tumor bearing dogs undergoing chemotherapy and marrow transplantation were also performed. The micro culture technique was standardized.

Plan:

Screening of patients and canine experimental subjects for lymphocyte dependent antigen reactive sites is crucial for any program of active matched transfusion and transplantation. The effect of chemotherapy on these tests will also be monitored.

Publications:

None

Project No. Z01 CM 06820-02 PO
1. Experimental Hematology Section
2. Pediatric Oncology Branch, MO
3. Bethesda, Maryland

PHS-NIH
Individual Progress Report
July 1, 1974 through June 30, 1975

Project Title: Blood Component Transfusion and Bone Marrow Transplantation

Previous Serial Number: NCI-3415, NCI-6301, NCI-3426

Principal Investigators: Robert G. Graw, Jr., M. D. and Geoffrey P. Herzig, M. D.

Associate Investigators: Malcolm I. Bull, M. D., Roger H. Herzig, M. D., Hans-Peter Lohrmann, M. D., Jean Charles Kauffmann, M. D., Bridgid G.Leventhal, M. D., David Poplack, M. D., N. Claude Gorin, M. D., Alois Gratwohl, M. D., William Penland, Gerhard R. F. Krueger, M. D., John Neefe, M. D., Joan M. Bull, M. D., Costan Berard, M. D., Harvey Gralnick, M. D., Jacqueline Whang-Peng, M. D., Mary H. McGinniss, Thomas C. Pomeroy, M. D., David Johnson, D. V. M., Paul I. Terasaki, Ph. D., David Dale, M. D., Charles H. Kirkpatrick, M. D., Charles Bowles, Ph. D., Gary Peck, M. D., Richard Simon, Ph. D., Regina Dowling, R. N., Ruth Estabrook, R. N., Joanne Beman, R. N., Louise Osborne, R. N., Denise Streeter, R. N., Shirley House, Ligita Novikovs, Frank Stout, Daniel Dean, Thomas Gray, James Hathorn, Lynne McKenzie, Rick Gonzales, Glenn Koteen, Cherie Freed.

Cooperating Units: Medicine Branch, NCI; Immunology Branch, NCI; Laboratory of Pathology, NCI; Hematology Service, Clinical Pathology, Clinical Center, NIH; Blood Bank, Clinical Center, NIH; Anesthesiology Department NIH; National Institute of Allergy and Infectious Disease, LCI; Department of Surgery, University of California at Los Angeles; Mary Imogene Bassett Hospital, Cooperstown, New York; Department of Medicine and Surgery, Division of Research Services, NIH; Plasmapheresis Unit, NCI; Dermatology Branch, NCI; Radiation Branch, NCI; Case Western Reserve, University School of Medicine, Cleveland Ohio; Armed Forces Radiobiology Research Institute, Bethesda, Maryland; Microbiological Associates, Bethesda, Maryland; Hazleton Laboratories, Vienna, Virginia; ABCOR Corporation, Boston, Massachusetts; Oakridge National Laboratories, Oakridge, Tennessee; Community Blood Service, Baltimore, Maryland (NIH Plateletpheresis Center).

Man Years:

Total: 12
Professional: 7
Other: 5

Project Description:

Objectives:

1) The provision of effective blood component transfusion therapy for patients with depressed bone marrow function, with emphasis on the development of improved procurement and transfusion techniques for blood, platelets and granulocytes.

2) Develop ablative chemo-radiotherapy protocols which can be used in conjunction with intensive supportive care for patients with diseases refractory to standard therapy.

3) Assessment of the methods for obtaining and maintaining allogeneic, autologous and isogeneic bone marrow grafts.

4) Study of patients with aplastic anemia in order to characterize the natural history of disease, response to chemotherapy and effectiveness of various forms of experimental therapy (intensive supportive care and bone marrow transplantation).

5) Examination of the effectiveness of combined modality treatment (chemotherapy, radiotherapy, immunotherapy and supportive care) in large animals with spontaneous neoplasms (canine lymphosarcoma) and to utilize preclinical animal models (monkey to dog) to investigate questions pertaining to supportive care and bone marrow transplantation (see 1, 2 and 3).

6) Evaluation of assays to characterize histocompatibility antigen systems which may influence transfusion responsiveness in patients alloimmunized to transplantation antigens, and tumor associated transplantation antigens as they may be detected with the same assays.

Methods Employed:

1) Transfusions of platelets and leukocytes in the Clinical Center, NIH, have continued to be the major means of evaluation of new transfusion techniques. Nine thousand five hundred donors who have been HL-A typed form the available donor pool. A contract facility - "NIH Plateletpheresis Center" has been functioning for 11 months for the plateletpheresis of selected donors thereby enlarging the capability for delivering selected (non-random) blood products for patients requiring platelet and granulocyte transfusions.

2) Efforts in allogeneic, isogeneic and autologous bone marrow transplantation have been continued for terminal leukemia patients, children with selected

solid tumors,and patients with aplastic anemia.

3) Preclinical animal model systems (primate and canine) have been utilized for testing transfusions, transplantation and experimental techniques for the combined modality treatment of spontaneous neoplasms.

Major Findings:

1) Blood Component Transfusion Support (Clinical Studies):

A. Platelets:

1. Transfusion: During the past fiscal year 130 patients at the Clinical Center and 23 patients from San Francisco, Boston, Milwaukee, Baltimore and five greater Washington area hospitals were transfused with HL-A selected four-unit platelet concentrates. To these 153 patients, 2,179 four-unit concentrates (8,716 units) were distributed: 1,905 to Clinical Center patients, 274 to non-NIH patients. The platelet procurement contract was awarded to Community Blood Services of Baltimore and the NIH Plateletpheresis Center was created on the reservation. The NIH Plateletpheresis Center during an 11 month period performed 2,769 procedures on 745 individual donors. The remainder of the plateletphereses were performed in the NCI Plasmapheresis Laboratory, as well as additional procurement studies using the Latham Blood Cell Processor. Seventy-two four-unit platelet collections were harvested in other blood centers-three European centers, two California centers and one Wisconsin center and were air-shipped to four patients at the NIH. A computer system has been devised and implemented to aid in the selection of HL-A compatible donors and also provides an ongoing clinical record of transfusion, transfusion reactions, responses and immunologic compatibility assays. This system permits integration of all important patient parameters (and research results) into the daily platelet selection process for each patient thereby increasing the safety and effectiveness of each transfusion.

2. Histocompatibility: The file of histocompatible (HL-A typed) donors has grown to 9,500 donors. Approximately 50 new individuals are HL-A and RBC typed each week. Blood was drawn from 2,840 prospective new donors for HL-A typing at the NIH Plateletpheresis Center during the contract period. Nine months ago, the National Cancer Institute implemented the "National Platelet Donor Pool", a computerized national blood component donor program. Currently six blood donor centers (Los Angeles, San Francisco, Seattle, Milwaukee, Chicago and Iowa City) have been incorporated into the donor pool from the National Cancer Institute for the purposes of sharing rare HL-A phenotype platelet concentrates among the participating institutions. Our current experience with the shipment of platelets from the National Cancer Institute as well as the platelets received from

other centers (see above) demonstrates the ease and feasibility of platelet exchange among medical centers. Continued investigation of histocompatibility variables which influence platelet donor selection reveals that HL-A typing remains an important first step in the selection process (platelets from 2 of 3 donors selected for HL-A give reliable post-transfusion increments and hemostasis); that the lymphocyte crossmatch with recipient serum fails to predict responsiveness to platelet transfusion; and that leukoagglutinins in a recipient's serum against leukocytes contaminating a platelet concentrate can inhibit platelet circulation and function. Newer assays under evaluation are: platelet aggregation, lymphocyte dependent antibody, serotonin release and a two step platelet antibody-macrophage assay (see 6).

B. Granulocytes:

1. Transfusion: Four hundred normal granulocyte transfusions have been given during the past year for suspected or proven gram-negative infection in granulocytopenia patients. The controlled randomized clinical trial of the efficacy of normal human granulocyte transfusions for the treatment of proven bacterial septicemia was continued for an additional twelve months. To date 26 patients with hematologic malignancies or solid tumors were stratified and randomized into the study (12 during the past 12 months). Two patients could not be randomized because of the lack of leukocyte procurement facilities. Five were not randomized at the discretion of the primary physician. Eight were not randomized since they did not satisfy protocol requirements for triple antibiotic therapy at the onset of suspected bacterial infection. Three patients were transfused with granulocyte transfusions on "local infection" protocol. Five patients were transfused with granulocytes for suspected infection as stipulated in the ablative "TEAM" protocol (see section 2). To date insufficient data have been collected concerning the utility of normal granulocytes in the prospectively randomized controlled trial; however, review of all normal granulocyte transfusions for patients with gram negative bacterial septicemia given at the National Cancer Institute between 1969 and 1974 reveals that the majority of patients experiencing recovery of bone marrow function during the transfusion period do not require granulocyte transfusions for recovery from infection. (89%-16/18 patients not given leukocyte transfusions recover with appropriate antibiotic therapy as contrasted to 100%-21/21 transfused with granulocytes in addition to appropriate antibiotic therapy.) In contrast patients who failed to recover marrow function during the transfusion period can be shown to benefit from repetitive granulocyte transfusion therapy ($\geq$ 3 transfusions). In that group 53%-8/15 recovered from bacterial septicemia when given granulocyte transfusions in addition to antibiotics when only 3%-1/30 recovered on antibiotics alone. Continued evaluation of the Filtration Leukapheresis technique has revealed: continued increased incidence of transfusion

reactions when cells are transfused from histocompatible donors; defective chemotaxis of cells is seemingly related to the duration of exposure of granulocytes to nylon fibers (this defect being partially corrected by pretreatment of donors with corticosteroids); increased stickiness of granulocytes as demonstrated by increased adhesion to second nylon fiber columns (as contrasted to cells collected with the Continuous Flow Blood Cell Separator and normal peripheral blood), this latter phenomenon being associated with thermostabile humoral factor which is present in the effluent from leukopaks during granulocyte collection and which imparts stickiness to normal third party granulocytes; and finally abnormalities in cell morphology and cell clusters during leukocyte collection as detected by scanning and transmission electron microscopy. Results of clinical trials employing Filtration Leukapheresis cells for the treatment of gram negative bacteremias indicate that four times as many granulocytes are required for the successful treatment of life-threatening bacterial infections when filtration cells are used as compared to cells from the NCI Cell Separator. The results of four serologic assays for the detection of antibody to transfusion donor cells are being correlated with post-transfusion increments and transfusion reactions. To date insufficient information is available concerning the utility of either of the assays although preliminary experience suggests that preformed leukoagglutinins against donor cells can inhibit the in vitro and in vivo function of transfused cells.

2. Preclinical Studies: Continued developments in centrifuge bowl design for the Continuous Flow Blood Cell Separator by engineers at the ABCOR Corporation and Oakridge National Laboratories have produced several bowls for preclinical testing. Currently an optimized centrifuge bowl for our use with the current cell separator is undergoing testing in the canine leukapheresis. An interface controller developed for the Cell Separator has been designed and is in clinical testing in the Plasmapheresis Laboratory at the NIH. This interface controller permits the optimum operation of the Continuous Flow Blood Cell Separator by controlling the input hematocrit to the instrument and maintaining the red cell plasma interface at a predetermined collection port.

   Preclinical studies examining the utility of granulocyte transfusion for the treatment of Pseudomonas pneumonia in dogs treated with 350 rads of total body irradiation revealed that cells collected with the Cell Separator and by Filtration Leukapheresis were both effective in eradicating gram negative bacterial pneumonias. Equal numbers of long-term survivors were produced in both groups although twice as many granulocytes were transfused to recipients given cells collected by Filtration Leukapheresis. Studies of post-transfusion increments following infusion of filtration cells were lower on a per cell basis than those following transfusion of Cell Separator cells; correlating with similar observations in humans. The

Pseudomonas pneumonia model has now been modified to study infusions of high titered Pseudomonas antibody in conjunction with antibiotics or antibiotics and granulocyte transfusions. These studies will also examine the schedule and interval between onset of infection and institution of transfusions necessary for a successful granulocyte transfusion. Pilot studies have studied the effect of antibody in the recipient dog's serum against cells transfused for documented infection (analogous to alloimmunized multiply transfused patients with hematologic malignancy). It is anticipated that these studies will be continued in order to study the consequences of incompatible leukocyte transfusions.

2) Ablative Chemo-Radiotherapy Protocols:

In an effort to provide effective therapy for patients with hematologic malignancy and solid tumors refractory to conventional therapy a combination chemotherapy protocol employing the agents currently used for bone marrow transplantation was devised. It was theorized that in the event a patient could be supported with blood component transfusions through ablative chemotherapy there was great likelihood that a significant long-term anti-tumor effect could be achieved. This was thought possible since patients treated with combination chemo-radiotherapy and transplanted with bone marrow have achieved extended remission and it is unknown whether remission duration should be attributed to the preparative chemo-radiotherapy or the effect of allogeneic reconstitution. The primary agent in the initial ablative chemotherapy protocol was cyclophosphamide (CYT), (45mg/kg/dose) used in conjunction with cytosine/arabinoside (ara-C) and 6-Thioguanine (6TG). A nitrosourea (MECCNU) was added as in the B.A.C.T. protocol for bone marrow transplantation (see section 3). Five patients with acute leukemia have been treated. Each patient was given 1 or 2 cycles per therapeutic course. Patients in a good prognosis category (no infection or previous organ toxicity) are randomized to receive prophylactic granulocyte transfusions in an attempt to prevent or delay significant bacterial infections. The protocol consists of three days of CYT; 4-6 days of ara-C/6TG; and a single dose of methyl CCNU. One patient has recovered bone marrow function three weeks following the completion of chemotherapy and remained in remission for 8 weeks.

3) Bone Marrow Transplantation:

A. Clinical Studies:

Fourteen patients were transplanted with allogeneic or autologous bone marrow for the treatment of refractory leukemia, lymphoma or aplastic anemia. See table below.

BONE MARROW TRANSPLANTS

Pt	Dx	Protocol	Survival (days)	Cause of Death
1	ALL	CY/TBI/ALLO	100	Interstitial Pneumonia
2	ALL	BACT/ALLO	150	Liver Failure (Australia Ag+)
3	ALL	CY/TBI/ALLO	16	Candida Pneumonia
4	AML	CY/TBI/ALLO	63	Interstitial Pneumonia
5	AML	BACT/ALLO	3	Sepsis/Liver Failure
6	ALL	BACT/ALLO	42	MLC+/Liver Failure
7	ALL	BACT/ALLO	20+	N/A
8	Burkitt's	BACT/AUTO	2	Cardiac Failure
9	Burkitt's	BACT/AUTO	7	Cardiac Failure
10	Burkitt's	BACT/AUTO	56+	N/A
11	Histiocytic Lymphoma	BACT/AUTO	20+	N/A
12	Aplasia	CY-P/C-ALLO	30	Sepsis, GI Necrosis
13	Aplasia	P/C-ALLO	1	Pneumonia
14	Aplasia	P/C-ALLO	7+	N/A

One patient remains in long-term remission following transplantation with B.A.C.T. (BCNU, CYT, Ara-C/6-TG) chemotherapy for 3.5 years. A profound immunologic deficit described in last year's report has been completely corrected.

B. Related Studies and Conclusions:

As noted above the allogeneic bone marrow transplantation experience was complicated by hepatic and pulmonary toxicity. This was thought to result from the consequence of radiation induced hepatic-vascular damage and radiation/drug induced pulmonary toxicity. Consequently the protocol employing CYT and total body irradiation has been temporarily discontinued until more patients are treated with chemotherapy alone, in order to study the long-term sequelae of this preparative regimen. Fatal cardiac toxicity with high dose CYT has now occurred in four transplant patients. For this reason the B.A.C.T. chemotherapy protocol has been modified to a lower daily dose of CYT (37.5 mg/kg/dose). It is thought that the toxicity of CYT is enhanced by the combination with other agents, possibly the nitrosourea or Ara-C. Two patients treated with the lower dose B.A.C.T. have tolerated the protocol. Current studies will examine the immunosuppression and antitumor effect of this new protocol. A protocol incorporating procarbazine with high dose CYT has been selected for the preparation of aplastic anemia patients for transplantation since approximately 20% of aplastic anemia patients fail to engraft when prepared with CYT alone. This protocol was well tolerated by two patients.

4) Aplastic Anemia:

Five patients with aplastic anemia have been admitted for evaluation and management with intensive supportive care. This has consisted of treatment with androgen therapy. Four patients have been treated with etiocholanolone (in collaboration with Dr. Sheldon Wolff, NIAID). Two of 4 patients have recovered partial cellular reconstitution. As noted above two patients required experimental bone marrow transplantation since they were deemed treatment failures and were complicated by bacterial infections thought to be incompatible with long-term survival. This program which has been initiated within the past several months will attempt to evolve an understanding of the basic physiologic defect in aplastic anemia (hematopoietic stem cell defect versus micro-environment defect). A systematic evaluation of various treatments will be attempted in conjunction with intensive efforts with hematologic supportive care. These patients offer an unusual opportunity to monitor immune response against transfused blood products since they are immunologically normal when examined with conventional assays of humoral and cellular immunity.

5) Preclinical Transplantation Studies and Experimental Therapy-Spontaneous Canine Lymphosarcoma:

A. Transplantation Studies:

The preclinical transplantation studies were aimed at standardizing general bone marrow transplantation and tumor treatment model systems. Methods for storage of bone marrow were examined using marrow preserved in liquid nitrogen for varying periods of time as well as bone marrow at 4°C for seven days. The minimum dose of frozen and thawed nucleated bone marrow cells necessary to obtain engraftment was shown to be 0.25 x 10^8 kg, a dose already shown to be the minimal dose of cells required for engraftment with fresh bone marrow. Complete restoration of circulating elements with marrow stored for two months occurred by 40 days, i.e. PMN's reached 1,000 and platelets 100,000 per cu mm by day 40. The results of *in vitro* studies including trypan blue dye exclusion, electron microscopy, chromosome studies, total nucleated bone marrow cell count, 2 hour DNA synthesis and agar colony formation were in accordance with the success of the grafts *in vivo*. Results of short-term preservation experiments indicated that storage of marrow for one week at 4°C damaged 75% of the precursor hematopoietic cells (stem cells). It was impossible to engraft allogeneic bone marrow which had been preserved at 4°C for 7 days because of limitations on marrow cell dose procurement.

Studies begun last year identifying variables important for successful and reproducable engraftment of allogeneic bone marrow following preparation with total body irradiation were completed. Using a cobalt 60 total body irradiaton facility the toxicity of supralethal total body irradiation doses from 800-2,000 rads at 3 dose intensities (10, 49 and 100 rads per minute) were determined in 76 intensively supported radiation control animals. The LD 50 (5) for these dose intensities was respectively 1,650, 960 and 910 rads. Random donor allogeneic bone marrow transplants were performed in 83 animals to test bone marrow cell dose and immune suppression afforded by 800 rads and 1,000 rads total body irradiation at a dose intensity of either 10 rads per minute or 40 rads per minute. It was concluded that the therapeutic index of total body irradiation conditioning regimens is greatest at the lowest dose intensity and that 1,000 rads mid plane followed within 24 hours by 5 to 7 x 10^8 nucleated bone marrow cells per kg (a dose readily obtainable from living donors) affords a high percentage of engraftment with predictable lethal graft versus host disease. Clinical and liver function test parameters of graft versus host disease were correlated with histopathologic criteria for graft versus host disease.

B. Investigation of Combination Chemotherapy and Bone Marrow Transplantation in Lymphoma Dogs:

Thirty-two dogs have been admitted for treatment with chemotherapy and bone marrow transplantation. Seven animals were randomized to total body irradiation followed by autologous bone marrow reconstitution-median duration of remission 137+ days. The median of remission from the last dose of chemotherapy for animals randomized to no further therapy was 22 days, all animals relapsing with progressive adenopathy by day 90. Future studies will examine the role of post-transplant chemotherapy and immunotherapy.

C. Immunologic Studies:

These studies are being conducted to determine the cellular and humoral immune responses of normal and lymphosarcoma dogs treated with total body irradiation and various sources of bone marrow. In general animals with lymphosarcoma were noted to have a profound depression of cellular immune reactivity at diagnosis and for several months following treatment with total body irradiation and reconstitution with a bone marrow. It was observed that human and guinea pig red blood cells form spontaneous immune rosettes with canine peripheral blood lymphocytes. Standardization of the E and the EAC rosette assays for normal dogs was accomplished, which will now permit further characterization of T and B lymphoid markers in experimental animals.

D. Monkey Marrow Transplantation:

The monkey marrow transplantation model system was employed to establish the minimum engrafting dose of autologous and allogeneic bone marrow using 1,050 rads, total body irradiation. The autologous bone marrow transplantation system was used to correlate hematopoietic stem cell toxicity of antilymphocyte serum with that observed in *in vitro* marrow culture.

6) Study of Histocompatibility and Tumor Immunity:

A. An assay of leukocyte migration inhibition has been established and standardized in order to study tumor immunity and alloimmunization to blood transfusions. To date leukocytes from three patients who have been immunized to RAJI tissue culture cells failed to migrate in response to intact RAJI cells and solubilized RAJI cell membrane extracts.

B. Studies of direct cell mediated cytotoxicity (CMC) of normal individuals and patients with hematologic malignancy against lymphoblastoid cell lines and leukemia blasts were continued. Significant CMC against the RAJI cell line was demonstrated in 96% (100/104) of normals compared to 60% (37/62) patients. Impairment of CMC was most severe in patients with active disease, 11/29 (38%) positive compared to 22/33 (79%) positive in remission. Against acute leukemia blasts 48% (42/88) of normals demonstrated significant CMC compared to 8% (1/12) patients with active disease and 7/14 (50%) of patients in remission. Testing of sera from patients with active disease did not reveal the presence of blocking factors. Most recently a variety of cell separation technics have been used to establish the nature of the effector cell responsible for mediating cytotoxicity in this system. Preliminary results implicate B lymphocytes in contrast to classically described direct cytotoxicity which is T-cell mediated.

C. Studies of in vitro lymphocyte sensitization by mixed leukocyte culture are being pursued in patients undergoing allogeneic bone marrow transplantation in an effort to establish an assay for the prediction of marrow graft rejection or graft versus host disease severity.

D. Presently available assays of alloimmunization do not reliably predict response to platelet transfusions. Three new technics for the detection of anti-platelet antibodies have been evaluated: antibody dependent cell mediated cytotoxicity (ADCC or LDA), stimulation of granulocyte hexose monophosphate shunt activity by antibody-coated platelets and phagocytosis of radiolabelled platelets. The first two technics were found to be technically unsatisfactory, but the platelet phagocytosis technic was successfully adapted for the demonstration of platelet alloantibodies. The assay appears to be quite sensitive and additional studies are in progress to determine possible clinical correlations.

Proposed Course of Project:

1) Continued evaluation of improved procurement and transfusion techniques for platelets and granulocytes. Of particular interest is the evaluation of donor selection using histocompatibility criteria and the relative effectiveness of leukocytes collected using various techniques.

2) Continued evaluation of granulocyte antigen typing in prospective donors for leukocyte transfusions.

3) Continuation of bone marrow transplantation studies emphasizing autologous bone marrow following preparation with chemo-radiotherapy.

4) Preclinical studies will be concerned with: Granulocyte transfusions into alloimmunized recipients; combined modality therapy of spontaneous canine lymphosarcoma; use of antilymphocyte serum as an immunosuppressant in conjunction with bone marrow transplantation.

5) Histocompatibility studies will continued to examine the relationship between serologic determinants (HL-A) and LD determinants (MLC) with assays measuring alloimmunization.

Keyword Descriptors:

Blood component transfusion therapy, ablative chemo-radiotherapy protocols, allogeneic, autologous, isogeneic bone marrow grafts, aplastic anemia, experimental therapy, combined modality treatment in large animals with spontaneous neoplasms, utilize preclinical animal models, histocompatibility antigen systems, transplantation antigens, tumor associated transplantation antigens .

Honors and Awards:

Robert G. Graw, Jr., M. D.

1) NCI member-Committee on Immunobiology of Cancer, 1974-1975.

2) Member NIH Committee on Transplantation Research, 1974-1975.

3) Board of Trustees, Medical Advisory Board-Leukemia Society of America, Greater Washington Area, 1975.

4) Pediatric Hematology and Oncology Subspecialty Board, 1974.

Invited Lectures:

Malcolm I. Bull, M. D.	10
Geoffrey P. Herzig, M. D.	4
Roger H. Herzig, M. D.	4
Robert G. Graw, Jr., M. D.	14

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5) Graw, R. G., Jr., Lohrmann, H-P., Bull, M. I., Decter, J., Herzig, G. P., Bull, J. M., Leventhal, B. G., Yankee, R. A., Bleyer, W. A., Buja, M., McGinniss, M. H., Whang-Peng, J., Gralnick, H. R., Krueger, G. R. F. and Henderson, E. S.: Bone Marrow Transplantation Following Combination Chemotherapy-Immunosuppression (B.A.C.T.) in Patients with Acute Leukemia. Transplantation Proceedings, 6: 349-354, 1974.

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Project No. Z01 CM 06830-05-PO
1. Pediatric Oncology
   Branch
2. Section on Infectious
   Disease
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: 1) Studies on Molecular Mechanisms of Productive Infection and Carcinogenesis Associated With DNA and RNA Tumor Viruses

2) Infectious Complications of Malignancy: Prevention and Therapy

Previous Serial Number: NCI-6830

Principle Investigator: Arthur S. Levine, M.D.

Other Investigators (this Section): Cephas Patch, Ph.D.; Sisir Chattopadhyay, Ph.D.; Ian Magrath, M.D.; Gilbert Jay, Ph.D.; Einar Everitt, Ph.D.; Martin Oster, M.D.; Phillip Pizzo, M.D.; Dorothy Moore, M.S.; Janet Hauser, B.S.; Richard Robinson, M.D.; Yvonne Wewerka-Lutz, M.D.

Other Investigators (Cooperating Units): Andrew M. Lewis, Jr., M.D.; Wallace P. Rowe, M.D.; Thomas J. Kelly, Jr., M.D., Ph.D.; Sherman Weissman, M.D.; Heiner Westphal, Ph.D.; George Khoury, M.D.; James Rose, M.D.; Kurt Kohn, M.D.; Herbert Reynolds, M.D.; Ronald Elin, M.D.; Edward Scolnick, Ph.D.; and Phillip A. Sharp, Ph.D.

Cooperating Units: LVD, NIAID; LCI, NIAID; LBV, NIAID; LMG, NICHD; LMPH, NCI; VLL, NCI; Division of Human Genetics, Yale University; Department of Microbiology, John Hopkins University; and Center For Cancer Research, Mass. Inst. of Tech.

Man Years:

Man Years:	Total:	28*
	Professional:	18
	Other:	10

*Does not include administrative or paramedical services.

Project Description:

Objectives:

1) To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.

2) To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control viral replication, transcription, translation, and maturation.

3) To improve the production and purification of Epstein-Barr virus (EBV) DNA and to use the reagent to explore the molecular mechanisms of infection and oncogenicity associated with this human herpes virus. Such mechanisms will be approached with the techniques previously applied in this laboratory to SV40 and Ad2.

4) To analyze the state and expression of the endogenous AKR murine leukemia virus genome in rodents with high-, low-, or no incidence of leukemia. A further objective is to explore the evolution, transmission and relation to leukemo-genesis of this virus, using cells, from a variety of rodents and non-rodents indigenous to widespread parts of the world.

5) To develop and evaluate the utility of various techniques and procedures to reduce the incidence of severe infection in patients with neoplastic diseases and in other compromised hosts; to improve methods by which occult infectious agents may be detected and eradicated; to evaluate the human acquired germ-free state and to learn the influence of this state on normal physiologic and metabolic processes.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use.

This laboratory routinely employs equilibrium density centrifugation, analytical ultracentrifugation, electron microscopic heteroduplex mapping, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromatography, and the _in vitro_ synthesis of viral DNA probes. Nucleotide sequencing of specific viral RNA transcripts is performed in collaboration with Dr. Sherman Weissman. _In vitro_ protein synthesis using cell-free systems is being introduced to the laboratory currently. An extensive tissue culture facility is maintained.

Quantitative evaluation of the efficacy of prophylactic techniques in compromised hosts is based upon comprehensive sampling of the patients and their environment for various micro-organisms, while such patients are offered a comprehensive antimicrobial regime in a laminar air-flow isolation-filtration unit.

Major Findings:

1) _Hamster cells transformed by nondefective Ad2-SV40 hybrids_. We have previously isolated and characterized five nondefective Ad2-SV40 hybrid viruses (Ad2$^+$ND$_1$-Ad2$^+$ND$_5$) which contain different but overlapping segments of the SV40 genome and small (2-5%) Ad2 deletions. Ad2$^+$ND$_1$ induces SV40 U antigen; Ad2$^+$ND$_2$, U and TSTA; and Ad2$^+$ND$_4$, U, TSTA, and SV40 T-antigen. Ad2$^+$ND$_4$ contains 43% of the SV40 genome, primarily the "early" region (associated with transformation). The SV40 segments have been mapped in relation to the wild-type SV40 genome by heteroduplex microscopy. Each of these hybrid viruses (as well as non-hybrid Ad2) transforms hamster kidney cells and such cells are oncogenic in newborn hamsters. The ND4HK1 transformed line contains SV40 T and TSTA, and SV40 RNA. The ND2HK2 line contains SV40 RNA but no detectable SV40 antigens; the other hybrid transformed lines contains no SV40 RNA. We have now studied the state and expression of the Ad and SV40 nucleotides within these hybrid-transformed lines in an attempt to understand their variation in viral gene expression. In particular, we explored the site of viral DNA integration, the quality of the incorporated viral genome, transcription and translation of specific regions of the hybrid genome, and various combinations of these processes. We find that by reassociation kinetics, the equivalent of 1-2 Ad2 genomes per cell are present within the Ad2 and hybrid-transformed hamster cell lines. It is notable that transformed hamster lines, which are oncogenic, have approximately the same content of Ad2 DNA as rat cells transformed by Ad2, which are not oncogenic. Transcription of the Ad2 moiety within the hybrid-transformed lines differs from Ad2 transcription in Ad2-transformed lines; these differences in the extent and orientation of transcription may reflect the SV40 insertion or the Ad2 deletion in the input hybrid genomes. Post-transcriptional processing of viral RNA occurs in both Ad2 and hybrid-transformed lines. In certain of the hybrid-transformed lines, SV40 DNA in the input viral genome has not been integrated; where the SV40 piece has been integrated, it is fully transcribed from the appropriate strand, yet this RNA is not fully

translated or not translated at all. All of these results indicate that interactions between the genomes of the nondefective hybrid viruses and the hamster cells they transform are random in some circumstances and non-random in others. The findings may be applicable to recombinant DNA experiments in general, i.e., not all heterologous DNA introduced into eukaryotic or prokaryotic cells will be integrated; of that DNA which is integrated, some may not be transcribed, or if transcribed, may not be translated.

2) A "new" early Ad2 RNA species in cells lytically infected by Ad2$^+$ND$_1$ and Ad2$^+$ND$_3$. We have studied cells lytically infected by nondefective Ad2-SV40 hybrid viruses to learn whether the SV40 insertion or Ad2 deletion in these hybrids would alter Ad2 or SV40 transcription from the hybrid genomes. We found that 1000 bases of Ad2 DNA at the $3'$ end of the SV40 DNA segment are expressed as cytoplasmic RNA in early Ad2$^+$ND$_1$ and Ad2$^+$ND$_3$-infected cells, but this transcript is not found during "early" wild-type Ad2 infection. The expression of this "new" early RNA species may be the result of (1) the segment of Ad2 DNA deleted from the hybrid genomes containing a transcribed sequence normally responsible for degradation of this "new" chain, (2) a mutation in the Ad2 DNA which creates a new RNA polymerase promoter, or (3) the $3'$ end of this particular RNA chain, which is coded by SV40, interacts with an Ad2-coded RNA sequence at the $5'$ end of the hybrid message, and this interaction stabilizes the intervening RNA chain during processing.

3) Structure and expression of two defective Ad2-SV40 hybrids which contain the entire SV40 genome. Two defective Ad2-SV40 hybrids (Ad2^{++}HEY and Ad2^{++}LEY) have been isolated which contain the entire SV40 genome. Upon infection of cells permissive for SV40, both hybrids give rise to infectious SV40, but with markedly different efficiencies. In the case of Ad2^{++}HEY, nearly all cells infected with the hybrid particle yield SV40 progeny, whereas with Ad2^{++}LEY, infectious SV40 is produced in only about one in 10^4 cells infected with a hybrid particle. We found by electron microscopic heteroduplex mapping that the Ad2^{++}HEY hybrid virus DNA contains significantly more than one SV40 genome and the extra SV40 DNA is arranged in a tandem repetition. Ad2^{++}LEY on the other hand contains approximately one SV40 genome. It is proposed that Ad2^{++}HEY yields infectious SV40 with significantly higher efficiency than Ad2^{++}LEY because the probability of an intra-molecular recombination event which results in excision of an intact SV40 genome is greater. We have also demonstrated the synthesis of nonhybrid SV40 DNA in Ad2^{++}HEY but not in Ad2^{++}LEY-infected cells. Both "early" and "late" SV40 RNA is transcribed efficiently in Ad2^{++}HEY-infected cells, but only early SV40 RNA is transcribed efficiently in Ad2^{++}LEY-infected cells. Ad2^{++}HEY induces SV40 U, T, and V antigens during lytic infection while Ad2^{++}LEY induces SV40 U and T only. These variations in the behavior of Ad2^{++}HEY and Ad2^{++}LEY regarding expression of SV40 functions probably reflect differences in the rate of SV40 excision from the hybrid genomes. The differences in the state and expression of integrated SV40 DNA displayed by Ad2^{++}HEY and Ad2^{++}LEY suggest possible mechanisms which underly major differences between SV40 lytic infection

and transformation.

4) AKR murine leukemia virus (MLV) genome: frequency of sequences in DNA of high-, low-, and non-virus yielding mouse strains. Studies with s single-stranded DNA probe complimentary to the RNA of mouse-tropic AKR MLV indicate that the complete genome of the AKR MLV is present in the DNA of high- and low-virus yielding mouse strains, while DNA of nonvirus-yielding strains contains only a part of the genome. Furthermore, in those strains where the genome is complete, 2 populations of virus-specific DNA sequences can be identified (more abundant and less abundant species) according to their rate of association with the probe. Low-virus-yielding mouse strains contain fewer copies of the less abundant species and consequently fewer complete viral genomes than do high-virus-yielding strains. Thus, in the ten strains tested, there is a good correlation between completeness of the genome of the AKR-type MLV in cellular DNA and the capacity of the cells to release infectious AKR-type MLV. Moreover, the number of complete viral genomes correlates with the frequency of infectious virus production by virus-positive strains. DNA from the wild mouse contains viral sequences identical to those of the non-virus producing laboratory strains, but DNA from the mouse indigenous to Kyushu Island (Japan) contains the complete AKR MLV genome. Proof has been obtained that one mouse-tropic virus-inducing locus in AKR mice, AKV-1, contains viral genetic material by demonstrating that AKR-type viral DNA sequences correlate completely with the presence of AKV-1 in mice which segregate for this locus. Finally, we have found that when cells from non-virus-yielding strains are infected with AKR MLV, they gain the population of less abundant sequences (and the number of copies of these sequences) that are normally associated with high-virus-yielding strains. However exogenous infection of AKR cells does not add additional sequences beyond those present endogenously.

5) A model of Ad2 DNA replication. Of the total intracellular virus-specific Ad2 DNA labeled during a one-hour pulse, about 20% was single-stranded. This DNA shifted predominantly to the double-stranded fraction during an extended chase incubation, suggesting that it represented single-stranded DNA in replicating intermediates. Furthermore, the single-stranded DNA annealed nearly equally to both strands of the Ad2 genome. These findings are consistent with a model of DNA replication involving strand displacement with synthesis of the displaced strand being initiated from either end of the parent template..

6) Production and purification of EBV DNA. The initial year's work with this virus has been primarily concerned with increasing the yield of virus from various cell lines and obtaining a pure and well characterized viral DNA. Various manipulations of the environment of cultured cells have induced a significant increase in a late viral-associated antigen which appears to be associated with a similarly increased yield of viral DNA. Modifications of methods for virus DNA purification have yielded a significantly purer EBV DNA than others have reported.

7) The limulus lysate (LAL) test in the detection of endotoxemia: lack of clinical utility. Reports of the clinical value of the LAL test for the detection of endotoxemia in man are discordant. In this study, 237 different plasma samples for the LAL test were obtained. The samples were drawn with concomitant blood cultures from 111 patients, the majority of whom had hematologic malignancies and almost all were febrile. Each plasma sample was tested with seven different LAL preparations from six different laboratories. We found a large number of positive blood cultures with negative LAL tests and vice versa, and despite a positive correlation with gram-negative bacteremia, neutrophilia, and elevated serum alkaline phosphatase, our studies suggest a limited clinical usefulness for the LAL test in the detection of endotoxemia.

8) Use of a Pseudomonas Aeruginosa Vaccine in Acute Leukemia and Cystic Fibrosis. A heptavalent lipopolysaccaride pseudomonas vaccine was evaluated in 22 acute leukemia patients and 12 cystic fibrosis patients during an 18 month interval. Thirty-two of 34 patients had an excellent serum hemagglutinating (HA) antibody response to immunization. In comparison to cystic fibrosis patients, the leukemia patients had a smaller HA antibody response, which lasted a shorter period of time, and also experienced greater toxicity from the vaccine. The mixing of adrenal corticosteroids with vaccine greatly decreased side reactions among leukemia patients without significantly inhibiting antibody production. Previous anti-neoplastic chemotherapy had little influence on antibody response in leukemia patients, with the exception of methotrexate. Subjects with leukemia had one pseudomonas infection out of 14 bacterial or fungal infections, while two pseudomonas infections occurred in a control group of 20 acute leukemia patients. Of the twelve patients with cystic fibrosis, four experienced pseudomonas infection after vaccination.

9) Rodent and human gnotobiology studies and their relation to cancer chemotherapy. We and others have shown that the incidence of fatal infection can be reduced in leukemia through the use of a comprehensive prophylactic antimicrobial regimen and isolation in a sophisticated laminar air-flow type of isolation-filtration unit. However, infection still occurs in these circumstances, and even when it does not, a certain fraction of subjects remains resistant to cancer chemotherapy. In current studies at NIH and under contract, we are attempting to develop improved prophylactic techniques in animals and man, and to determine the value of reduced infectious incidence in a variety of tumors. During the past year, M.D. Anderson Hospital investigators have made the following observations (Contract No. N01-CM-12094) on chemotherapy of solid tumors with the protected environment - prophylactic antibiotic program (PEPA): twelve evaluable patients with sarcomas (Sa) and 13 with malignant lymphomas (ML) were randomized to receive 3 courses of initial chemotherapy with or without the PEPA. Patients with Sa were treated with the combination of cyclophosphamide, vincristine, adriamycin and dimethyltriazeno imidazole carboxamide. Patients with ML were treated with the combination of cyclophosphamide, adriamycin, vincristine, prednisone, and bleomycin. Doses were escalated by about 30% during each subsequent course regardless of the degree of myelosuppression

if no severe infection occurred during the previous course. Among the 7 Sa patients treated with PEPA, there were 5 partial remissions (PR), and 2 stable disease (SD). Among the remaining 5 Sa patients treated without PEPA there were 3 PR, 1 SD and 1 failure (F). Dosage was escalated in 6 of 7 with PEPA and 2 of 5 without PEPA. The frequency of severe infection was 14% and 20% respectively. Among the 7 ML patients treated with PEPA, there were 3 CR and 4 PR. Among the remaining 6 ML patients treated without PEPA, there were 3 CR and 3 PR. Dosage was escalated in 5 of 7 patients with PEPA and 4 of 6 without PEPA. The frequency of severe infection was 14% and 33%, respectively. These preliminary results indicate that the PEPA may be useful in solid tumor chemotherapy.

In rodent studies at the University of Notre Dame (Contract No. N01-CM-23718), it was found that all detectable horizontally transmitted microorganisms were eliminated from conventionally-reared AKR mice by continuous feeding of 4 nonabsorbable antibiotics for 12 or more weeks, plus total body immersion in a germicidal solution. When the decontaminated mice developed spontaneous lymphatic leukemia each was treated for the disease by an immunosuppressive drug (cyclophosphamide). The treated leukemic decontaminated mice remained microorganism-free. They survived longer, and in better condition, than cyclophosphamide-treated conventional leukemic AKR mice. Moreover, germ-free (GF) AKR mice were administered 1000 R X-rays (whole-body) and 24 hours later they were injected iv with viable bone marrow cells from GF DBA/2 mice. The chimeric GF AKR mice appeared healthy and did not develop spontaneous leukemia up to age 15 mo when the experiment was terminated. Conventional AKR mice with bone marrow cells from conventional DBA/2 mice died of GVH disease. Leukemia appeared spontaneously in conventional AKR mice transplanted with AKR bone marrow cells. Thus, leukemia was prevented in the GF AKR mice with allogeneic chimerism.

Significance to Bio-Medical Research and the Program of the Institute.

Our studies of nondefective Ad2-SV40 hybrids have made a significant contribution to knowledge of the genetic organization and expression of the two tumor viruses during lytic infection and oncogenesis. Moreover, these hybrid virus studies offer insight into the state and expression of integrated heterologous DNA in general, and must be considered in planning the type of "genetic engineering" experiments possible with the recombinant DNA technology. The phenomenon of tandem repetition of animal virus integrated DNA, demonstrated for the first time in our studies of Ad^{++}HEY and Ad2^{++}LEY, may be the critical determinant of whether integrated viral DNA remains integrated in a eukaryotic cell and induces transformation, or whether this DNA is excised and induces lytic infection. In studies on the AKR murine leukemia virus (in collaboration with Dr. Wallace P. Rowe), our objective is to correlate specific leukemia virus sequences with specific biological events (e.g., leukemogenesis). Comparative study of the genetic information specified by different MLV sequences in different strains of mice is now feasible and we believe that it will be possible to determine the evolutionary origin and transmission of MLV infections. Our studies during the past year have explained why only some mice produce virus and develop leukemia even though all mice may contain MLV antigens. Moreover it has been established that a major mouse chromosome locus for the production of MLV is in fact composed of MLV nucleotide sequences and is not merely a host locus which permits the expression of remote viral functions. It should be emphasized that these studies concern a known naturally occurring animal leukemia virus within the context of known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not yet been characterized and where the genetics of the disease are not known and cannot be controlled. Our EBV studies are important because study of the molecular mechanisms displayed by this virus have been retarded by the failure to produce significant quantities of the virus and of purified viral DNA. We believe that our accomplishment in this regard will permit a wide variety of experiments on the molecular biology of this human virus in lytic infection and in tumorigenesis. Studies on the prevention and treatment of infectious complications in malignancy are of importance because a significant number of cancer patients appear to die of infection prior to achieving the best results that extant cancer therapy may offer.

Proposed Course:

(1) We shall employ various biochemical techniques to distinguish between the viral sequences specified by different endogenous murine leukemia viruses in the DNA of mice with differing expression of MLV information. DNA from other rodent species will be tested for homology with various MLV DNA probes, and these studies should speak to the evolutionary origin, transmission, completeness, and expression of the MLV genome.

(2) Studies on the nondefective Ad2-SV40 hybrid viruses will emphasize the kinetics of specific SV40 transcripts early and late in infection. We shall also employ specific SV40 transcripts in in vitro protein synthesizing systems, to generate tumor-associated proteins. We shall continue our studies on the effects of SV40 insertion and Ad2 deletion on the regulation of expression of integrated DNA. We shall attempt to determine whether we can transform cells using the isolated SV40 DNA segments from those hybrid viruses which contain nearly all of the "early" SV40 region.

(3) We have begun a comparative study of in vitro transcription of Ad2 DNA by mammalian DNA dependent-RNA polymerase and E. coli RNA polymerase. We shall determine which areas of the Ad2 genome are transcribed by the two enzymes.

(4) We are attempting to identify and isolate certain virus-specified phosphorylated proteins synthesized during productive Ad2 infection. We hope to learn whether these proteins are involved in regulatory steps in viral DNA replication and/or transcription or whether they are of importance in virion assembly. We shall also explore whether viral DNA-protein complexes (core structures) bear a precursor-product relationship to fully assembled virions.

(5) Attempts are being made to isolate transcriptional complexes associated with cellular DNA during the early stage of productive infection of monkey cells by SV40. The objective of this work is to determine whether early SV40 messages are transcribed from integrated or free DNA.

(6) Human adenoviruses are unable to grow in primary monkey cells unless those cells are co-infected with SV40. Studies are underway to determine the mechanism of this phenomenon ("enhancement"). One hypothesis is that the block in the development of adenovirus is the result of a translational defect in which adeno-specific RNA, although made in large quantities, is unable to specify the attachment of ribosomes. If we learn that Ad2 RNA is completely transcribed in primary monkey cells but not associated with ribosomes, we will then attempt to translate this RNA in a cell-free system using isolated early SV40 proteins as possible translation co-factors. Little is known about the control of translation in eukaryotic cells, and the SV40-Ad2 enhancement system may be an appropriate model with which to explore the control of translation in animal cells.

(7) The relation of specific areas of the EBV genome to known biological activities will be studied in various cultured lymphoblastic lines and in human tumor biopsy materials. We shall also be concerned with the molecular identification of different EBV strains and how specific nucleotide sequences may influence the different biological activities of these strains. The site and nature of the association of EBV DNA with host DNA will be explored in infectious mononucleosis and in African and American Burkitt's tumor.

(8) We shall begin a quantitative evaluation of laminar air-flow rooms and prophylactic antibiotics in the treatment of early-stage lymphomas and leukemia which are resistant to conventional therapy, and in bone marrow transplantation. Studies under contract will continue (including a new study at L.A. Childrens' Hospital of rhabdomyosarcoma and neuroblastoma, NO1-CM-53831). In collaboration with the EORTC, we are beginning a multi-center study of empiric anti-bacterial and anti-fungal agents in patients with fever of unkown origin. We will continue studies of the limulus lysate test, pseudomonas vaccine, adenine arabinoside (an anti-viral agent), and initiate clinical trials of poly I· poly C/poly-l-lysine (a stabilized polynucleotide resistant to serum hydrolysis) as an anti-tumor agent.

Key Word Descriptors:

1) SV40
2) Adenovirus 2
3) Ad2-SV40 hybrid viruses
4) EB virus
5) AKR murine leukemia virus
6) nucleic acid hybridization
7) viral oncogenesis
8) gnotobiology
9) laminar air-flow rooms

Honors and Awards:

Arthur S. Levine, M.D.

1. Visiting Lecturer, Cold Spring Harbor Animal Virus Workshop, July, 1974.
2. Chairman, Symposium on Infectious Complications in the Compromised Host, International Congress of Pedatrics, Buenos Aires, October, 1974.
3. Speaker, American Cancer Society/National Cancer Institute-National Conference on Advances in Cancer Management Part I, New York City, November, 1974.
4. Chairman, Symposium on Infections in Cancer Chemotherapy, Annual Meeting of the EORTC, Brussels, January, 1975.
5. Discussant, National Academy of Sciences-Asilomar International Conference on Recombinant DNA Molecules, California, February, 1975.
6. Invited Speaker, Symposium on Ovarian Carcinoma, The Massachusetts Medical Society, Springfield, February, 1975.
7. Invited Speaker, Grand Rounds, UCLA School of Medicine, Los Angeles, March, 1975.
8. Discussant and Co-editor of Proceedings, International Workshop on Interferon in the Treatment of Cancer, New York City, March, 1975.
9. Visiting Professor, Univ. of Minnesota Depts. of Medicine and Pedatrics, May, 1975.

Sisir Chattopadhyay, M.D.

1. Invited Speaker, Cold Spring Harbor Symposium on Tumor Viruses, June, 1974.
2. Invited Speaker, Gordon Conference on Animal Cells and Viruses, July, 1974.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH CENTER

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1974 - June 30, 1975

During the past year, the Baltimore Cancer Research Center relocated its clinical activities to the ninth floor of the University of Maryland Hospital, where it continues its In and Outpatient care activities along with the Clinical Research Pharmacy Service, Protective Environment Unit, Microbiologic Surveillance and Cell Component Therapy. Temporary renovations have allowed continued high-level patient care and research, while more definitive renovations and refurbishing are being planned and carried out. The activities of the Laboratory of Pharmacology have continued at the U.S.P.H.S. Hospital location in Baltimore, until the completion of the adjacent laboratory building into which these activities can move at the University of Maryland Hospital. Despite the effects of translocation and separation, a high-level of patient care, applied and basic research have been maintained.

Phase I studies of IV-CCNU and intrathecal thiotepa have been completed and Phase II studies are in progress. Azacytidine in the treatment of acute adult non-lymphocytic leukemia has been identified as an effective agent in this disease. IV-MeCCNU in metastatic sarcoma, thiotepa, vincristine and CCNU in renal cell carcinoma, cis-diamine dichloro platinum in testicular tumors and IV-MeCCNU and epipodophyllotoxin (PTG) in brain tumors are continuing Phase II studies.

The anthracyclines, daunomycin and adriamycin, continue to be used alone and in combination in conjunction with studies of pharmacologic disposition and metabolism. Adriamycinol has been identified as one major metabolite of adriamycin mediated through a specific cytoplasmic enzyme. Four human metabolites of daunomycin have been characterized and several others have been isolated. The metabolism of CCNU by hydroxylation on the cyclohexyl ring has identified five metabolites by use of high pressure liquid chromatography. The major portion of human plasma nitrosourea has been identified as 4-hydroxyl CCNU.

Combined modalities therapy studies in Hodgkin's disease and malignant glioma have resulted in significant prolongation of disease-free survival. Protective environment (laminar air flow) and detailed microbiologic and viral diagnostic support have provided a better understanding of the environment and ways in which they may be enhanced in the immune suppressed host. Intergrated programs in patient care, including the BCRC Nursing Service, Cell Component Therapy, Infectious Disease Control and Clinical Research Pharmacy Service have led to a singularly unique multidisciplinary approach to total patient care. Clinical, pharmacologic, microbiologic and therapeutic data have been coordinated and reduced through use of sophisticated computer resources and a special pilot study of the Problem-Oriented Medical Record System (PROMIS) is being pursued.

The biologic markers of malignant disease, such as muramidase, alpha fetoprotein, carcinoembryonic antigen (CEA), Regan isoenzyme, and plasma and urinary polyamines are being studied in relation to diagnostic and therapeutic activities. The concentration of spermadine in human urine appears to be of particular significance in evaluating disease state. A strong correlation between the presence of polyamines and RNA synthesis appears to be mediated through RNase activity and polyguanylic and polyadenylic acids acting as competitive inhibitors may be dissociated from the enzyme surface by spermidine. A human plasma ribonuclease, capable of binding the polyamines, may add further modulation to this entire complex system.

More basic studies have indicated abnormal ribonculease activity in the precancerous mouse, thus pointing toward possible predictors of neoplastic transformation. The time-effect relationship of polyadenylic-polyuridylic acid in prolonging survival and preventing leukemia in the AKR mouse may indicate an immunoenhancing effect. Cell membrane, in association with purified rubeola virus, is needed to provide the best antigenic source for template recognition and lymphocyte responsiveness. In addition, complement appears capable of mediating specific cross-sensitivity to antigenic determinants of tumor, as well as normal cells.

Additional laboratory studies are evaluating specific endogenous tissue growth substances, methods of purifying and isolating phytohemagglutinin lectins and the polarity of viral RNA with respect to the section of the polymer coding for reverse transcriptase.

The Baltimore Cancer Research Center's intergrated programs of laboratory and clinical research continue to participate within the academic community by close working relatioships with the oncology efforts of the University of Maryland and Johns Hopkins University. BCRC physicians and scientists provide consultative and collaborative foci for individual studies at many levels, as well as participating in major teaching hospitals within the greater Baltimore community. Through such collaborative efforts, patient referral, and discussions at all levels of the scientific program, provide the nucleus for continued ongoing multidisciplinary efforts. It is anticipated that the Laboratory of Pharmacology will transfer to Howard Hall II, currently under construction at the University of Maryland, during this fiscal year, and will realize an increased capability and productivity by its reassociation with the Clinical Branch. The renovations necessary for bringing the Clinical Service up to full complement should be completed this year. This will provide a continuum of care with the Outpatient Department, Inpatient beds and all Support Services in a through and through configuration on one floor. All of this will be immediately adjacent to the Laboratory of Pharmacology, so that a maximum of scientific interaction can be fostered.

Summary Report
Clinical Branch, Baltimore Cancer Research Center DCT, NC

July 1, 1974 through June 30, 1975

Progress in many areas has occurred during this fiscal year. Phase I study of I.V. CCNU has been completed and the results reported. Phase II study of intrathecal thio-TEPA in meningeal leukemia is in progress. Phase II trials of azacytidine and guanazole in adult acute nonlymphocytic leukemia have been completed with the result that azacytidine has been identified as a highly active agent in this disease. Phase II evaluation of thio-TEPA, vincristine, and CCNU in renal cell carcinoma continues. Phase II studies of I.V. methyl CCNU and other agents in metastatic sarcoma are also continuing. Phase II study of cis-diamine-dichloroplatinum in testicular tumor and azotomycin in metastatic sarcoma have been initiated. Phase III studies in acute nonlymphocytic leukemia, adult acute lymphocytic leukemia, disseminated Hodgkin's disease and non-Hodgkin's lymphoma, and metastatic testicular carcinoma continue to yield valuable data. Phase III studies in breast cancer have been initiated. Phase III studies of methyl CCNU and epipodophylotoxin (PTG) in brain tumors are encouraging.

Combined modality therapy studies in Hodgkin's disease utilizing surgery, radiation therapy, and combination chemotherapy have resulted in significant prolongation of disease-free survival. Chemoimmunotherapy trials with MER and levamisole in leukemia, lymphoma, melanoma, and oat cell carcinoma of the lung are in progress.

Participation in oncology programs at the two medical schools in Baltimore and in other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community the impact of the therapeutic advances resulting from the DCT program.

Protected environment (Laminar Air Flow) studies have been enhanced by detailed microbiologic surveillance and diagnostic support. This effort has led to an understanding of the microbiologic environmental balance in favor of the patient. Specific studies have examined Pseudomonas infection, colonization of the environment and improved culture taking and retrieval systems. Viral diagnostic surveillance is being studied in detail for its efficacy with respect to the management of the immune suppressed host. A Phase II study of cytosine arabinoside in Herpes zoster infection has been completed with the result that the disease is not altered by this therapy.

Results to date in this area indicate that adult acute nonlymphocytic leukemia patients treated in a protected environment have significantly fewer infections during treatment and survive significantly longer than those treated in a conventional environment.

Biologic markers of malignant disease such as muramidase, alpha fetoprotein, carcinoembryonic antigen, Regan isoenzyme, and plasma and urinary polyamines have been studied in detail and the data correlated with the type and extent of malignant disease.

This mass of data has been prepared for analysis and analyzed by a pilot computer-organized problem-oriented medical system. Doctors, nurses, pharmacists, and other related personnel that take part in patient care and clinical research contribute their input to the record through this computerized system.

New plans for the next fiscal year include Phase II and IV studies in colon cancer and breast cancer.

Project No. Z01 CM 06027-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Microbiological Services for Clinical and Research Studies of Cancer Patients.

Previous Serial Number: 6027

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S.; Robert C. Good, Ph.D.; Robert W. Chambers, M.D.; Arthur S. Levine, M.D.; Martin H. Cohen, M.D.

Cooperating Units: Medical Service, Baltimore Cancer Research Center, DCT, NCI, Baltimore, Md.; Pediatric Oncology Branch, DCT, NCI, Bethesda, Md.; Medical Oncology Branch, Veteran's Administration Hospital, Washington, D.C.; Hazleton Laboratory, Vienna, Va.; Viral Diagnostic Laboratory, Georgetown Univ., Washington, D.C.

Man Years:

Total:	4 1/2
Professional:	3
Others:	1 1/2

Project Description:

The objective of this project is to supply microbiologic diagnostic services of a high quality for the study of actual and potential infection of cancer patients with viruses, bacteria, mycoplasma and fungi. Further objectives are to study the patients' normal flora, food and environment in an effort to curtail acquisition of potentially pathogenic agents and supply support for evaluation of protective environments and various research investigations.

Bacteria, mycoplasma and fungal diagnostic cultures are performed by Hazleton Laboratories under Contract N01-CM-33705. The level of man hour effort of contract personnel was 25,855 hours at a cost of $374,902.00. A total of 14,355 specimens during the calendar year of 1974 were analyzed for aerobic, anaerobic and fungal flora, and all microorganisms recovered were speciated whenever possible utilizing standard procedures as well as serotyping and bacteriophage typing. Emphasis was placed on enhancement of recovery of anaerobes by use of a new anaerobic hood, recovery of *Mycoplasma* spp. through introduction of new media supplied by the contractor and on further evaluation of the development of resistance to commonly used antibiotics.

Viral diagnostic cultures and serologies are performed by the Viral Diagnostic Laboratory of Georgetown University under Contract N01-CM-12123 at a cost of $254,181.00. The level of man hour effort of contract personnel was 24,752 hours. There were a total of 6,516 samples studied during the calendar year, 809 of which were for viral isolation, 5,215 for viral antibody titer, 394 for RIA and 98 for electron microscopy. Viral recovery rates from diagnostic samples vary seasonally between 5 and 10%. Approximately 75% of the sera submitted for viral antibody titer determinations have a 4-fold change in titer to one or more of the 20 antigens tested. These fluctuations are under analysis to resolve problems of interpretation.

These contracted facilities provide accurate and current microbiologic data for support of cancer patients receiving chemotherapy. Aside from patient care, the data generated provide the microbiologic basis for a number of research protocols, current information on the resistance to antibiotics of organisms, on cross-infection and on various sources of environmental contamination. For example, these environmental cultures have determined that pasteurized fruit juices served to the patients contained large numbers of several *Candida* species and *Torulopsis glabrata*. Also, it was shown that insulating materials sprayed onto overhead beams at the University of Md. Hospital, where the BCRC moved, was heavily contaminated with *Aspergillus* species (niger, flavus & fumigatus) which undoubtedly provided a source of infection for cancer patients (see Project No. 06264). Extensive culturing is underway to determine if there are other possible sources of contamination in our new environment. These data are stored in a computer and are data retrievable for investigational purposes. (See Project No. 06039). The program will be continued along the same lines as previously. Additional food samples will be cultured to determine if food serves as a source of potential pathogens.

Keyword Descriptors:
1. Viral diagnostic cultures.
2. Cancer patient microbiologic flora.
3. Contracted diagnostic microbiology.
4. Fungal environmental contaminants.
5. Environmental cultures.
6. Cancer patient serum antibody screen.
7. Surveillance cultures for cancer patients.
8. Cancer patient normal flora.
9. Protective environmental microbiologic support.
10. Microbiologic research support program.

Honors and Awards: None

Publications:

Aisner, J., Sickles, E.A., Schimpff, S.C., Young, V.M., Greene, W.H., and Wiernik, P.H. *Torulopsis glabrata* Pneumonitis in Patients With Cancer. JAMA, Vol. 230, No.4, Oct. 28, 1974.

Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M. and Wiernik, P.H., Aspergillus Infections Among Patients with Cancer: An association with fireproofing materials in a new hospital building. JAMA (In Press)

Project No. Z01 CM 06039-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Computerized Microbiology Reporting and Data Retrieval Systems

Previous Serial Number: 6039

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: John W. Gerster, William L. Robinson, Richard K.C. Hsieh

Cooperating Units: Health Services Research, U.S. Public Health Service Hospital, Baltimore, Maryland.

Man Years:

Total:	1/2
Professional:	1/3
Others:	1/6

Project Description:

The objective of this project is to provide a rapid, accurate means of reporting preliminary and final results of microbiologic diagnostic data generated by a distantly located laboratory facility. The final results are permanently stored and the data are retrievable for use by clinicians and microbiologists to perform prospective and retrospective studies. The computer is utilized to summarize final reports to be placed in the patients' charts. Error detection systems and quality control for the microbiologic data, generated by the contracted laboratory, is provided by computer programs.

Patient data, dates, source of samples and requests are entered from the hospital into data files stored in a timesharing computer system. The distant contracting laboratory reviews this data prior to receipt of samples and assigns laboratory numbers. Results are entered as they become available and preliminary data is sent twice daily. As each report becomes final, it is batch processed at the hospital computer center and a final report is generated. The data is permanently stored (after processing through an error detection system) and is used as the base for data retrieval.

The patient census program (see Project Report CM 06220), which records patient vital statistics, rooms, beds, attending physician, diagnosis, etc., is used by the Microbiology Reporting System to detect errors in hospital numbers, names, etc. It also serves as a base for epidemiologic reports of various pathogenic organisms.

The data retrieval programs allow retrieval by various parameters for use in patient care, monitoring of endemic and epidemic infectious agents, and various research support efforts. Immediate review of total cultures of an individual patient or a particular species of organisms or the environmental organisms can be made. A report of unusual results for review and antibiotic susceptibility summary reports are provided on a regular basis. The preliminary reporting system has been reviewed during the year and a revised system, which will increase efficiency and flexibility, is in preparation.

Keyword Descriptors:
1. Microbiology data retrieval.
2. Computerized microbiology reporting system.
3. Preliminary microbiology reporting system.
4. Microbiology data storage.
5. Microbiology error detection system.
6. Computer assisted epidemiologic review.
7. Antibiotic susceptibility summary reports.
8. Patient microbiology summary reports.
9. Contracted microbiology computer reports.
10. Endemic flora computer detection.

Honors and Awards: None

Publications: None

Project No. Z01 CM 06219-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Cancer Patient Microbial Flora: Acquisition, Colonization and Infection.

I. Determination of prevalence of organisms recovered from various sites of cancer patients.

II. Studies of new or unusual organisms recovered from cancer patients.

III. A study of *Proteus* spp. colonization and clinical significance in cancer patients.

IV. A study of *Serratia* recovered from cancer patients.

V. Acquisition of yeasts during hospitalization.

VI. Combined antibiotic therapy for the treatment of cancer patients with infection.

Previous Serial Number: 6219

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Robert C. Good, Ph.D., Philip Hartman, Ph.D.

Cooperating Units: Hazleton Laboratories, Vienna, Va., and the Department of Biology, Johns Hopkins University, Baltimore, Maryland.

Man Years:

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

The objective of this project is to study the microbial flora of the cancer patient, i.e., to determine acquisition of microflora, colonization and subsequent infections. The prevalence of organisms which are acquired from the hospital and subsequently became part of the patients' flora is continuously monitored by routine surveillance cultures of the high risk patients. Such microorganisms more frequently cause infection than the patients' original flora. Any new or unusual organisms recovered from patients are studied extensively by all available microbiological methods (assimilation techniques included) to determine their classification and pathogenic potential. Proteus spp. are continuously monitored by a modification of the Craddock-Watson test for bacteriocin production to subspeciate them for surveillance purposes. Genetic changes in the original set of Proteus test strains made it necessary to select a number of new test strains which are under evaluation for their stability. A set of Serratia antisera has been purchased to allow serotypic identification of these organisms. The Serratia recovered from patients are now under investigation with this antisera. The infection of cancer patients by yeasts and their possible acquisition from environmental sources is being investigated. Approximately 90% of the cancer patients have 1 to 4 species of yeasts recovered from samples during their hospital stay. Almost half of these yeasts were grown from 1 or both of 2 sets of admission surveillance cultures. Slightly over half were recovered from subsequent cultures and defined as having been acquired. Over half of the acquired yeasts eventually were recovered from the infectious processes or autopsy cultures, whereas only 1/3 of yeasts present in admission cultures occurred similarly. Candida albicans was recovered the most frequently, but C. tropicalis was more prone to cause bacteremias. Possible sources were dietary products (particularly fruit juices), hospital rooms which became contaminated in the presence of "shedders", hand lotions and colonized sink drains and bath tubs. Patients who are placed on the combined antibiotic therapy protocol are monitored for infecting organisms. Also, studies are in progress to determine the influence of cancer chemotherapeutic agents combined with antibiotics on organisms that commonly cause infection. All of these studies help to define more precisely the organisms which infect patients, and provides the basis for protection of the patient against potential pathogens in the environment. It is planned to continue these investigations.

Keyword Descriptors:
1. Surveillance cultures.
2. New or unusual organisms.
3. Infection.
4. Bacterocin typing of Proteus spp.
5. Serratia serotyping.
6. Acquisition of yeasts.
7. Candida tropicalis bacteremia.
8. Combined antibiotic therapy.
9. Cancer chemotherapy influence on antibiotics.
10. Acquisition of microflora.

Honors and Awards: None

Publications: None

Project No Z01 CM 06223-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on *Pseudomonas aeruginosa* in a Cancer Research Center.

I. *Pseudomonas aeruginosa* in cancer patients and its effect on colonizations and infections.

II. The effect of genetic and environmental factors on susceptibility to antibiotics and epidemiology of *Pseudomonas aeruginosa*.

III. Incidence of *Pseudomonas* species other than *P. aeruginosa* as potential infectious agents in cancer patients.

IV. The host immune response to *Pseudomonas aeruginosa* and its role in resistance to infection.

Previous Serial Number: 6223

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D., Maureen Morris, B.S., Norman R. Friedman, B.S.

Cooperating Units: None

Man Years:

Total:	2
Professional:	1 1/2
Others:	1/2

Project Description:

Prevention of infection is important in the overall management of cancer patients. The study was undertaken to evaluate the incidence of *P. aeruginosa* types in these patients as etiologic agents of infections, to investigate the influence of extrinsic factors such as the patient environment on characteristics of the organism, to monitor the infectious potential of other *Pseudomonas* spp. and to explore the host immune response to the organism in order to provide insight into mechanisms by which *P. aeruginosa* infections can be eliminated.

All *Pseudomonas* spp. were speciated by a schemata devised in this laboratory. Strains of *P. aeruginosa* were serotyped by antisera based on "O" specificity. Antimicrobial susceptibility was determined by disc, tube, and agar plate dilution techniques. Phenotypic and genotypic changes in the microorganism was determined by standard bacterial genetic procedures. Immunoglobulin levels to *P. aeruginosa* in patients' sera was established by microhemagglutination. Studies of the B and T lymphocytes from immunized and nonimmunized mice were determined by incorporation of radiolabelled DNA and RNA precursors during lymphocyte transformation.

P. aeruginosa was recovered from 26% (199) of 762 cancer patients. Recovery rates were highest in ANLL (53%) and ALL (51%) as compared to chronic leukemias (27%),myelomas (30%), solid tumors (25%) and lymphomas (14%) ($P < .001$). The incidence in CNS patients was higher than in the other solid tumor patients ($P < .04$). The total number of types isolated was greatest in ANLL and least in lymphoma patients even when compared to serotypic recovery in noncancer patients ($P < .01$). Type 3 was more likely to be recovered from lymphoma patients ($P < .01$) and Type 6 from solid tumor patients ($P < .01$). Types 4 and 7, which were frequently isolated in the cancer population, were rarely recovered from noncancer patients, ($P < .01$). ANLL and ALL patients (highest colonization rate) were most often colonized by Type 7 ($P < .05$) which also colonized CNS patients frequently, whereas Type 6 colonized ANLL patients as well as Hodgkin's, non-CNS solid tumor and non-cancer patients ($P < .05$). Type 4 frequently colonized ALL and non-Hodgkins' lymphoma patients, Type 3, non-CNS solid tumor patients and Type 2, myeloma patients. Recovery of *P. aeruginosa* from the blood was highest in leukemic patients ($P < .01$) (the strains were most often Types 2 and 7), while the recovery from urine was significantly greater in solid tumor ($P < .01$) and noncancer ($P < .01$) groups. Although there was no statistical difference in recovery from sputa, Type 3 demonstrated a predilection for the sputa of leukemic and lymphoma patients. Sixty-five percent of the cancer patients acquired *P. aeruginosa* ($P < .001$). The acquisition rate was significantly higher among leukemic patients. Type 7 was the most frequently acquired strain (P .004), whereas Type 2 was most commonly present at admission (P .01). Leukemic patients from whom Type 4 was isolated at admission rarely became colonized or infected with these strains (P .0042). Colonization and infection rates were statistically higher with acquired strains; however, once colonization occurred, regardless of whether the strain was present at admission or acquired, over 65% of the patients became infected. Acquired strains of Types 1 and 7 caused more infections than did the other types. Immunoglobulin levels to *P. aeruginosa* were highest in solid tumor patients and lowest in lymphoma patients. Following urinary tract infections and pneumonias, Ig level increases to the causative type were noted. Among solid tumor patients, the average level was higher for those patients from whom *P. aeruginosa* was not recovered or who were colonized, whereas it was higher in leukemic patients who were neither colonized nor infected. While the level of the lymphoma group was lower overall, lymphoma patients from whom *P. aeruginosa* was not recovered had the highest average titer, and those patients who were colonized or infected rarely had titer levels above 8.

As *P. maltophilia* and *P. cepacia* are frequently recovered from patients and their environs and are highly resistant to most antibiotics, the efficacy of trimethoprim-sulfamethoxazole as a therapeutic drug choice was investigated. All *P. cepacia* were inhibited by 2 μg/ml or less of trimethoprim, whereas all

P. maltophilia were resistant to greater than 32 μg/ml. Both species were susceptible to the drug combination; the average zone of inhibition (disk test) was larger for P. cepacia ($P < .005$).

Segments I and II has been completed, but monitoring of these organisms will be continued. Segments II and IV are under study.

Keyword Descriptors:

1. Pseudomonas aeruginosa.
2. Serotypes colonizing and infecting.
3. Immune response.
4. Development of resistance.
5. Phenotypic and genotypic alteration.
6. Prevention of infection.
7. Antibiotic susceptibility testing.
8. Lymphocyte transformation.
9. Serotypic predilection for sites.
10. Prevalence of serotypes according to cancer type.
11. P. maltophilia and P. cepacia trimethoprim-sulfamethoxazole susceptibility.

Honors and Awards: None

Publications:

1. Young, V. M. and Moody, M.R., Serotyping of Pseudomonas aeruginosa. J. of Inf. Dis. Supp. 130:S47-S52, 1974.

2. Moody, M.R. and Young, V.M., In Vitro Susceptibility of Pseudomonas cepacia and Pseudomonas maltophilia to Trimethoprim and Trimethoprim-Sulfamethoxazole. Antimicrob. Agents and Chemother. In Press.

3. Schimpff, S.C., Greene, W.H., Young, V.M., and Wiernik, P.H. Significance of Pseudomonas aeruginosa in the Patient with Leukemia or Lymphoma. J. Inf. Dis. Supp. 130:S24-S31, Nov. 1974.

4. Greene, W.H., Moody, M., Hartley, R., Effman, E., Aisner, J., Young, V.M., and Wiernik, P.H., Esophagoscopy as a source of Pseudomonas aeruginosa sepsis in patients with acute leukemia: the need for sterilization of endoscopes. Gastroenterology 67:912-919, 1974.

Project No. Z01 CM 06254-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques.

I. Studies of the relative effective areas of different culture media and techniques for the isolation and identification of specific species of microorganisms.

II. Studies of antibiotic susceptibility involving new methodology or newly developed antimicrobial agents.

Previous Serial Number: 6254

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S.; Maureen J. Morris, B.S.; Robert C. Good, Ph.D. and Donna S. Purvis, B.S.

Cooperating Units: Hazleton Laboratories, Vienna, Va.

Man Years:

Total:	1/3
Professional:	3/12
Other:	1/12

Project Description:

The objective of this project is to evaluate and implement new methodology whenever it offers an advantage to recovery of microorganisms from cancer patient samples and to develop and standardize methods for performing accurate susceptibility tests for new antibiotics as they become available.

Chocolate agar with and without the incorporation of 12 I.U. of Bacitracin were compared for the recovery rate of Hemophilus spp. In a 12-month study, 225 sputa were inoculated onto the 2 media. Hemophilus spp. were recovered from 77 samples (34%) using the selective medium, but only 28 (12%) on plain chocolate agar. H. parainfluenzae was isolated most frequently (20%), followed by H. influenzae (9%), H. parahemolyticus (7%) and H. hemolyticus (4%). Recovery for 2 species from the same sample was observed in 13 specimens (6%). Suppression of the normal gram positive and gram negative cocci in the sputum allowed more growth of Hemophilus spp. without inhibiting gram negative rods or fungi and occasionally allowing more rapid recovery. This selective medium is now utilized for all sputum cultures.

The API Enteric Strip has been claimed to be useful for rapid speciation of saccharolytic pseudomonads. Ten strains each of *Pseudomonas aeruginosa*, *P. maltophilia*, *P. fluorescens*, and 9 strains each of *P. cepacia* and *P. putida* were utilized to evaluate the strip. *P. aeruginosa* and *P. maltophilia* had a correct identification rate of 70%. Half of the *P. fluorescens* strains were placed in a group which included *P. fluorescens*, *P. aeruginosa* and *P. putida* and the other half were incorrectly identified. No specific identification of *P. putida* is possible with this system and only 44% of them were correctly placed in the above group. None of the *P. cepacia* could be identified. In total, 25 of 56 strains were incorrectly identified. This system is not adequate for speciation or grouping of the clinically important pseudomonads. Specific identification is important as the antibiotic susceptibilities of these organisms differ among the species.

At present, the use of Czapek agar for recovery of fungi is being evaluated. It has been noted that Aspergillus lung infections are generally preceded by colonization of the upper respiratory tract with these organisms. A recent episode of increased number of aspergillus infection led us to a study of this medium for possible enhanced detection.

Pharyngitis and pneumonia persist as frequent minor or major complications during the therapy of acute lymphocytic and nonlymphocytic leukemia. As approximately half of the severe pharyngitis cases and a number of pneumonias have not yielded causative organisms, in spite of repeated cultures, new methods for collecting and growing *Mycoplasma hominis* have been implemented and are under evaluation.

A report in the literature on the variability in Ca and Mg content of antibiotic susceptibility testing media influencing gentamicin susceptibility test results has led us to a study of this problem. Lots of media tested confirmed the variability and inadequate amounts of these ions present. The Ca and Mg content of the media has been adjusted to recommended levels and a number of previously tested strains are being re-evaluated to determine possible changes in MIC of the gentamicin. This antibiotic is one of those most commonly used for infection in cancer patients.

Keyword Descriptors:

1. Chocolate bacitracin agar for Hemophilus.
2. API Enteric Strip.
3. Saccharolytic pseudomonad identification.
4. Czapek agar.
5. Aspergillus infections.
6. Cultivation of Mycoplasma.
7. Gentamicin susceptibility tests.
8. Ca and Mg in susceptibility test media.
9. Selective media for *Hemophilus*.
10. Misidentification of pseudomonads.

Honors and Awards: None

Publications: None

Project No. Z01 CM 06938-01 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Environmental Studies.

I. Environmental sources of microflora potentially pathogenic for hospitalized patients.

II. Food and plants as possible sources of potentially pathogenic microorganisms.

III. Decontamination of drains.

IV. Investigation of products used in the care of cancer patients.

V. Evaluation of protective environments for the support of cancer patients' receiving chemotherapy.

Previous Serial Number: None

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Norman R. Friedman, B.S.; Robert C. Good, Ph.D.; Marcia R. Moody, M.S.

Man Years:

Total:	1 1/3
Professional:	1/3
Other:	1

Project Description:

The objective of this project is to survey organisms found in the environment, on plants, in the air, in food and water, in products used in the care of cancer patients, and on personnel who work with them, for the presence of potential pathogens. Means are sought to eliminate such organisms. Methods used are Rodac "touch" plates, air samples taken with a Microban Air Sampler, swabs for sink drains, etc. and Millipore filter systems. Food is weighed, liquified in an Osterizer and quantitative and qualitative cultures run. Unclean areas have been found in the environment and the areas decontaminated. Repairs have been made on the ice machine and new methods introduced for its use to prevent re-contamination. A study is underway to determine the best way to decontaminate drains. The use of Chlorox was unsuccessful; original microflora was again present in large numbers at the end of 2 hours. Draino eliminated bacteria for a number of hours, but allowed overgrowth with Candida spp. Other methods are under evaluation. Yeasts have also been found in abundance in iced tea, lemonade and fruit juices. These items have been eliminated or pasteurized products introduced to replace them. Yeasts and bacilli were found in carboxymethylcellulose, a product which was to be introduced as a carrier for mouth disinfectants. It was determined that some samples of milk and milk products with high Bacillus counts were out-dated and usage of out-dated milk was eliminated. Further emphasis will be placed on defining environmental contamination problems in the new location of the Baltimore Cancer Research Center and studies of food will be intensified to determine the extent to which food may provide a source of hospital-acquired microflora. Personnel are regularly cultured to discover carriers of potentially pathogenic microorganisms.

Various protective environments are also under evaluation for their use in protecting the patient from acquisition of bacteria from the hospital. An initial evaluation of a "Med-Assist" air filtration system, which consists of two HEPA filters with fans with adjustable flow rates, are provided for each end of a patient's bed. The positive pressure HEPA filter unit at the head of the bed passes air to the negative filter unit at the foot of the bed. The positive unit filtered air is pulled through the negative unit and directed down a plenum at the base of the unit which directs the returning air stream beneath the bed to the head unit to be recirculated. The units are mobile and can be pushed into any patient room where a relatively laminar air flow environment across the bed would be desirable. Extensive sampling of 5 patients and environment have shown that thus far no patient has acquired gram negative rod flora from the environment. Furthermore, there was a clearly demonstrated reduction of air-borne organisms. Rodac contact plates of various surfaces revealed no patient-connected flora. A current study is designed to compare the effectiveness of protective environments afforded by laminar air flow rooms, which have been shown to reduce total infection, Med-Assist air filtration systems in combination with complete reverse isolation and complete reverse isolation with no air filtration. (See Project No. 06217).

Keyword Descriptors:
1. Hospital acquisition of microflora.
2. Environmental microbiological surveillance.
3. Sources of contamination.
4. Laminar air flow rooms.
5. Med-Assist air filtration systems.
6. Reverse isolation procedures.
7. Culture of food products.
8. Decontamination of sink drains.
9. Fruit juices as a source of yeasts.
10. Protective environments.

Project No. Z01 CM-06217-06 BCRC
1. BCRC
2. Medical Oncology Section
3. Baltimore, Md.

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Prevention of Infections in Patients with Cancer.

Previous Serial Number: NCI-6217

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: Peter H. Wiernik, M.D.

Cooperating Units: Viola Mae Young, Ph.D. - Research Microbiology Section
Clarence L. Fortner, M.D. - Patient Care Pharmacy
Lois Jepsen, R.N. - Nursing Service

Man Years

Total:	7
Professional:	4½
Other:	2½

Project Description

Infection is a major cause of serious morbidity and the major cause of death at this Center. Patients with acute leukemia tend to become infected with hospital-acquired organisms such as *Pseudomonas aeruginosa*; those with mycosis fungoides are susceptible to *Staphylococcus aureus* acquisition and infection, and patients with lymphoma are prone to develop *Herpes zoster*. The following programs have been designed to evaluate methods for reducing infectious complications among these patients with the intent of improving the quality of survival and increasing the chance for complete tumor remission.

A) Basic Infection Prevention Measures - Based on previous investigations, the following procedures have become standardized: 1) urinary catheters, indwelling intravenous catheters, and hyperalimentation are avoided except under extenuating circumstances. 2) All intravenous bottles and tubing are replaced daily; butterfly needles are changed every other day. 3) Staff members apply a quaternary ammonium lotion to hands on a routine basis. 4) Disposable sitz baths are utilized. 5) All adult patient with acute leukemia a) ingest nonabsorbable oral antibiotics every four hours b) eat only a cooked food diet c) utilize hexaclorophene, iodine, and quaternary ammonium compounds daily for skin and/or oral hygiene. Although infections are still common among patients with advanced cancer, the overall incidence has been steadily reduced and certain types have been markedly reduced, especially gram-negative bacillary infections.

B) Protected Environments - Patients with acute nonlymphocytic leukemia are randomly assigned to 1) a regular room, 2) a regular room with complete reverse isolation, or 3) a regular room with reverse isolation plus air filtration. Patients are all treated equivalently with regard to antileukemia therapy, supportive care, and other infection prevention techniques (e.g., oral nonabsorbable antibiotics.) This study is designed to determine the potential value of reverse isolation with or without air filtration as an adjunct to infection prevention.

The present study was initiated in October, 1974 and has now included ten patients. Documented infections have been uncommon in each experimental group and no infectious deaths have occurred. Insufficient patients have been evaluated to assess whether the addition of isolation or air filtration is of added usefulness.

C) Prevention of Staphylococcus aureus Infection - Patients at this Center who become colonized with Staphylococcus aureus and who then become predisposed to infection (e.g., skin lesion necrosis in mycosis fungoides or granulocytopenia in acute leukemia) are the patients who most commonly develop infection with this organism. Therefore, the colonized patient who will be at high risk of developing infection is treated prophylactically for 5 days with rifampicin and cloxacillin. Rifampicin is used to kill S. aureus residing intracellularly and cloxacillin is used for the major reservoirs found extracellularly. At the present time, this project has insufficient data to reach firm conclusions.

D) Herpes zoster Prevention - Zoster tends to occur in specific subgroups of lymphoma patients (# 6264) following exposure to other people with Varicella-zoster infections. Although infected inpatients are isolated immediately, infection continues to occur suggesting a need for further preventive measures such as zoster immune globulin and/or transfer factor.

Keyword Descriptors: Infection prevention of cancer.

Publications: (1974-1975)

Schimpff, S.C., Greene, W.H., Young, V.M., et al.: Infection prevention in acute nonlymphocytic leukemia - Laminar air flow room reverse isolation with oral, nonabsorbable antibiotic prophylaxis. Ann. Intern. Med. 82: 351-358, 1975.

Project No.: Z01 CM 06230-06 BCRC

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

Previous Project No.: Same

Principal Investigators: Peter H. Wiernik, M.D., John A. Levi, M.D.

Other Investigators: John Sutherland, M.D., John A. Singer, M.D.
Malcolm Cooper, M.D., Larry Kvols, M.D.

Cooperating Units: Pathology, Tumor Surgery, and Radiology and Nuclear Medicine Departments, University of Maryland Hospital

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

Accurate determination of extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Analysis of 150 patients with Hodgkin's disease who have undergone staging laparotomy and splenectomy has revealed that pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of the cases. Prediction of splenic involvement by Hodgkin's disease was incorrect in 1/3 of the patients, approximately 1/3 of abnormal lymphangiograms could not be confirmed at laparotomy, and a number of cases of involvement of nodes in the porta hepatis region outside the usual radiation therapy ports were discovered. Occult hepatic involvement was discovered in some cases in whom liver function tests and physical examination were normal, and others suspected of having liver involvement were shown to have disease confined only to lymph nodes. These findings altered therapeutic approaches significantly in many patients.

It was discovered that patients presenting with supradiaphragmatic Hodgkin's disease had occult disease limited to the spleen, splenic hilum and celiac axis nodes in most cases in which abdominal tumor was documented at laparotomy. Extranodal involvement was not seen in any of the 85 patients with supradiaphragmatic clinical stage I or II Hodgkin's disease, and most patients would have had all apparent disease adequately treated had extended field radiation therapy been administered without performing staging laparotomy. Therefore, we are currently randomizing patients who present with nodal disease above the diaphragm who do not have the systemic "B" symptoms of Hodgkin's disease to undergo extended field radiation therapy on the basis of their clinical stage without performing laparotomy, or alternatively to undergo surgical staging with modification of therapy based on the documentation of abdominal Hodgkin's disease. Although a number of patients have been entered on this study, it is too early for evaluation at this time.

Patients who present with a suspicion of abdominal involvement undergo laparotomy prior to the initiation of therapy because of the relatively high risk of involvement of nodal sites outside standard radiation therapy ports, or extra nodal involvement, particularly of the liver.

Keyword Descriptors: Hodgkin's disease, staging

Honors and Awards: None

Publications:

1. O'Connell, M., Schimpff, S., Kirschner, R., Abt, A, Wiernik, P.: Epithelioid Granulomas in Hodgkin's Disease - A Favorable Prognostic Sign? JAMA (in press).

2. Schimpff, S., O'Connell, M., Greene, W., and Wiernik P.: Infections Following Splenectomy in Ninety-two Patients with Hodgkin's Disease: A Clinical Review. Amer J Med (in press).

3. Schiffer, C., Levi, J, Wiernik, P.: The Significance of Abnormal Circulating Cells in Patients with Hodgkin's Disease. Brit J Haematol (in press).

Project No.: Z01 CM 06259-04 BCRC
1. BCRC
2. Medicine Section
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Extension of the Computerized Problem-Oriented Medical Information System (PROMIS) into the Cancer Treatment Area.

Previous Serial Number: Same

Principal Investigator: Robert J. Esterhay, Jr., M.D.

Other Investigators:
Paul Chang, M.D.
William R. Grove, Pharmacist
William L. Robinson, Research Programmer
Carole A. Rulenz, A.R.T.
Peter H. Wiernik, M.D.
Michael D. Walker, M.D.
Viola M. Young, Ph.D.
Lois Jepsen, R.N.
Clarence L. Fortner, Pharmacist

Cooperating Units:
PROMIS Laboratory
Department of Medicine
University of Vermont
Buringling, Vermont 05401
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Cooperating Investigators:
Lawrence L. Weed, M.D.
George E. Nelson, M.D.
Jan R. Schultz, Research Associate
Stephen V. Cantrill, Research Programmer
Keith G. Morgan, Research Programmer

Man Years:		
	Total:	3
	Professional:	3
	Other:	0

Project Description:

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device with electrostatic printer similar to those in use in Burlington, Vermont, where the computer is located. BCRC has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.
2. The basic components of the remote terminal at the BCRC are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, Digiscribe, and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.
3. The BCRC staff has developed antineoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. Currently over 20,000 displays have been developed by the PROMIS group.
4. Problem-specific display plans for acute non-lymphocytic leukemia and non-Hodgkin's lymphomas have also been developed which includes a series of protocols for treating these specific problems. The protocol displays couple the user to the protocol specific information and ordering drug display sequences. The problem formulation displays for chronic lymphocytic leukemia and Hodgkin's disease are under development at this time. In addition, displays for problem oriented reporting of bone marrow findings are being developed.

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem-Oriented Medical Record. The problem defining sequence determines the specific plan, and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific antineoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user can couple learning with doing while gathering information and treating patients' cancer problems.

Keyword Descriptors: computer, problem-oriented, medical information system, cancer, treatment, patients, displays

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06263-04 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

Previous Project No.: Same

Principal Invstigator: Peter H. Wiernik, M.D.

Other Investigator: John A. Levi, M.D.

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

1. To assess the value of surgical staging in patients with apparently localized lymphomas.
2. To determine whether adjuvant chemotherapy will improve treatment results in patients with localized lymphomas treated with radiation therapy.

Patients with malignant lymphomas which are apparently clinically localized (stage I or stage II) undergo staging laparotomy to detect occult foci of intra-abdominal tumor. Patients with negative laparotomy findings (pathological stage I or II) are then randomized to receive either a) radiation therapy alone or b) radiation therapy followed by 6 courses of combination therapy utilizing cytoxan, vincristine, and prednisone. Patients who are found to have at least stage III disease are treated with combination chemotherapy on a different protocol.

Several patients with apparently localized supradiaphragmatic malignant lymphoma have been found at laparotomy to have occult disease below the diaphragm which would not have been properly treated had local therapy been given on the basis of clinically apparent disease only. Eight patients whose staging laparotomies were entirely negative were randomized to radiation therapy with or without adjuvant chemotherapy. Relapses have been seen in both study groups. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in treating localized malignant lymphomas.

There is evidence that truly localized malignant lymphomas are radiocurable if high dose radiation is given to all sites of involvement. On the other hand, such approaches would not be expected to be effective in patients with disseminated disease. Employing staging laparotomy as an additional diagnostic test to determine the extent of disease, therefore, may allow better patient selection for attempts at curative radiation therapy. In addition, it should be determined whether the addition of adjuvant chemotherapy will significantly prolong disease free and overall survival.

Keyword Descriptors: Non-Hodgkin's lymphoma, staging

Honors and Awards: None

Publications:

1. Levi, J., O'Connell, M., Murphy, W., Sutherland, J., Wiernik, P.: Role of ^{67}Gallium Citrate Scanning in the Management of Non-Hodgkin's Lymphoma. Cancer (in press).

Project No. Z01-CM-06264-06 BCRC
1. BCRC
2. Medical Oncology Section
3. Baltimore, Md.

PHS - NCI
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title:	Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients.
Previous Serial Number:	NCI - 6264
Principal Investigator:	Stephen C. Schimpff, M.D.
Other Investigators:	Joseph Aisner, M.D., Sheldon Landesman, M.D. A. Collier Smyth, M.D., Peter H. Wiernik, M.D.
Cooperating Units:	Research Microbiology, Viola M. Young, Ph.D., Norman Friedman, M.D.

Man Years

Total:	2½
Professional:	2
Other:	½

Project Description

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Similar details are obtained on infections detected at the time of postmortem examination.

A) Overall Clinical Infections - Review of all infections occurring among all patients treated for cancer by a small number of physicians under protocol conditions on a single hospital floor over a 5-year period has provided a unique opportunity to detect differences in infection type and incidence among different classes of tumor and to better understand the predisposing factors to each class of infections.

B) Infections Documented at Autopsy - All infections documented at autopsy during the past 5 years are presently under review. Results thus far clearly indicate that fungal infections (especially *Candida* spp, *Torulopsis glabrata*, and *Aspergillus* spp) are a major cause of infectious death and until the past year were rarely diagnosed before death.

C) Pneumonia - The use of transtracheal aspiration and bronchial brushing techniques has resulted in a major increase in the premortem identification of the etiologic agents of pneumonia. The ability to promptly define the etiologic agent has allowed use of specific therapy at an early date with resultant improved response rates and diminished toxicity. (See report # 6913).

D) Hepatitis. Of 59 consecutive acute nonlymphocytic leukemic patients (ANLL) admitted to the BCRC over a 2 year period, 36 lived more than 6 weeks and were studied for the incidence and characteristics of hepatic dysfunction. Despite screening of all blood products for HAA, 32/36 pts (89%) developed abnormal liver function tests seemingly unrelated to chemotherapy or disease activity. Only 3 of 21 patients who had frequent HAA assays determined by radioimmunoassay ever had positive HAA. The liver function test abnormalities were very prolonged in 63% and recurrent in another 19%. With the absence of staff infections, this markedly high incidence (89%) of frequently prolonged (63%) liver function test abnormalities among 36 patients with acute leukemia, generally HAA negative (86%) suggests a parenterally transmitted hepatitis that is not characteristic of hepatitis type A or B.

E) Aspergillus Infection - Recently, an increased occurrence of Aspergillus infections was seen making this infection the leading cause of infectious death at the BCRC. A careful microbiologic sampling of the environment revealed contamination of fireproofing materials in the building which is thought to represent the major reservoir in this hospital. The use of more aggressive diagnostic techniques in patients with pneumonia has allowed for earlier diagnosis and therapy resulting in complete response in 2 of 8 pts and clinical improvement in an additional 3 patients.

F) *Torulopsis glabrata* infection. A retrospective analysis of microbiology records, autopsy information and clinical records disclosed that *T. glabrata* has caused infection in 27 patients during the past 48 mos. A pulmonary portal of entry for this organism was suggested since 24/27 patients had primarily pulmonary involvement. Isolation of this organism from multiple sites on surveillance cultures was shown to correlate closely with subsequent clinical infection. *T. glabrata* infection is increasing in frequency and represents a significant contribution to infectious deaths among patients with advanced cancer.

Keyword Descriptor: Infection Diagnosis, Cancer Patients.

Publications:

Levine, A.S., Schimpff, S.C., Green, R.G., Young, R.C.: Hematologic malignancies and other marrow failure states: progress in the management of infections. Sem. Hematol. 11: 141-201, 1974.

Levine, A.S., Schimpff, S.C.: Antimicrobial therapy in the compromised host in antimicrobial therapy, 1974.

Schimpff, S.C.: Diagnosis of infection in patients with cancer. Europ. J. Cancer (in press).

Schimpff, S.C., O'Connell, M.S., Greene, W.H., Wiernik, P.H.: Infections in ninety-two splenectomized patients with Hodgkin disease - a clinical review. Am. J. Med (in press).

Schimpff, S.C., Bennett, J.E.: Abnormalities in cell-mediated immunity in patients with Cryptococcous neoformans infections. Allergy and Immunity (in press).

Greene, W.H., Moody, M.C., Hartley, R., et al.:Esophagoscopy as a source of pseudomonas sepsis in patients with acute leukemia. Gastroenterology 67, 912-919, 1974.

Aisner, J., Sickles, E.A., Schimpff, S.C., et al.:Torulopsis glabrata pneumonitis in patients with cancer. JAMA 230: 584-585, 1974.

Schimpff, S.C., Greene, W.H., Young, V.M., Wiernik, P.H.:Significance of Pseudomonas aeruginosa in the patient with leukemia or lymphoma. J. Inf. Dis. 130 (suppl): 524-529, 1974.

Project No. Z01 CM 06266-06 BCRC
1. BCRC
2. Medical Oncology Section
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Therapy of Infection in Patients with Cancer

Previous Serial Number: NCI-6266

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: Joseph Aisner, M.D., Peter H. Wiernik, M.D., Sheldon Landesman, M.D.

Cooperating Units: Patient Care Pharmacy, BCRC, Clarence L. Fortner, M.D.
Research Microbiology, BCRC, Viola M. Young, Ph.D.
Norman Friedman, B.S.;
Division of Infectious Diseases, University of Maryland,
Harold Standiford, M.D.:
European Organization for Research and Treatment of Cancer, Antimicrobial Project Therapy Group.

Man Years

Total: 1¼
Profession: 1
Other: ¼

Project Description

A) Prospective Randomized Controlled Trial of Three Bactericidal Antibiotic Combinations as Initial Empiric Antibiotic Therapy of Suspected Infection in Granulocytopenic Patients with Cancer. - The problems of broad spectrum antibiotic usage are formidable and include a high frequency of superinfections plus occasional renal damage. It was therefore decided to determine in an appropriate prospective, controlled trial whether gram-negative bacillary infections in granulocytopenic patients respond better to a penicillin-like drug (Carbenicillin or cephalothin), an aminoglycoside (gentamicin), or the synergistic combination (carbenicillin-gentamicin for *P. aeruginosa* or cephalothin-gentamicin for *Klebsiella* or *E. coli*). Because of the large numbers of patient trials required, the BCRC has joined with an 18 hospital group to study this question under the auspices of the European Organization for Research on the Treatment of Cancer.

The trial, begun Jan 3, 1974, has included over 125 BCRC patients which represents about 33% of the total of 315 patients trials in the EORTC trial. Each of the three combinations have proven to be very effective with a combined response rate of 63% for bacteremias and 95% for other microbiologically or clinically documented infections. The study continues as outlined.

B) Prospective, Randomized Controlled Trial to Test the Efficacy and Safety of Low-Dose Cytosine Arabinoside to Prevent the Progression of Herpes Zoster - Because Zoster infections at this cancer center are common and because they have a significant degree of morbidity, and because there is preliminary evidence to suggest that cytosine arabinoside will halt the multiplication of Varicella-zoster virus, it was decided to use cytosine arabinoside as systemic therapy of patients with early zoster in a controlled, randomized trial to determine whether this therapy would be better than placebo in halting the progression of infection. This prospective controlled investigation indicated that, although free of major toxicities, the use of cytosine arabinoside was of no value in halting local progression. This study was therefore discontinued.

C) Zoster Immune Plasma Therapy for Disseminated Herpes Zoster - Herpes Zoster progresses to widespread disseminated with pneumonia, encephilitis, glomerulitis, etc in certain cancer patients. Plasma with high tiers of antibody to the Varicella-zoster virus can be obtained from normal individuals after zoster or varicella infections and infused into patients with disseminated zoster in an attempt to check the spread of this infection.

Of eight patients with severe disseminated zoster or varicella pneumonia who received zoster immune plasma (ZIP), all but one had at least symptomatic improvement. The study will therefore be continued.

Keyword Descriptors: Infection Therapy, Cancer Patients

Publications:

Schimpff, S.C., Fortner, C. L., Greene, W.H., Wiernik, P.H.: Cytosine arabinoside for localized herpes zoster in patients with cancer: Failure in a controlled trial. J. Inf. Dis. 130: 673-676, 1974.

Kluge, R.M., Standiford, N.C., Tatem, B.,et al.: The carbenicillin-gentamicin combination against Pseudomonas aeruginosa. Ann.Intern.Med. 81: 584-587, 1974.

Project No.: Z01 CM 00275-06 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 - June 30, 1975

Project Title: Cell Transfusion Therapy

Previous Project Number: Same

Principal Investigators: Charles A. Schiffer, M.D., Joseph Aisner, M.D.
John H. Wolff, M.D., Peter H. Wiernik, M.D.

Other Investigators: Frances Sanel, Ph. D., Morton Schmucker, M.D.

Cooperating Units: Baltimore Red Cross, Community and Plasma Service, Dr. Paul Terasaki, University of California, Los Angeles, California; Dr. R. Rubin, Johns Hopkins School of Public Health, Baltimore, Maryland; Research Pathology Service, BCRC, Baltimore, Maryland

Man Years:

Total: 5
Professional: 2
Other: 3

Project Description:

Treatment of patients with cancer with aggressive chemotherapy and radiotherapy has produced an increased need for blood components to deal with the complications of bone marrow suppression. This program was designed to treat and prevent hemorrhage and infection in these patients.

I Platelet Transfusion - Platelets obtained from single units of whole blood are obtained from the Baltimore Red Cross while platelets obtained from two-unit plateletphereses are supplied by Community Blood and Plasma Services (CBPS). HL-A matched platelets for alloimmunized patients are provided by multi-unit plateletpheresis of related and unrelated donors performed at the BCRC and at CPBS. (HL-A typing is performed by Dr. P. Terasaki, Los Angeles, California.)

In the past year a Model 30 Blood Processor (Haemonetics Corp., Natick, Massachusetts) has been used to increase the efficiency of platelet and granulocyte procurement. Studies are underway to determine the precise bands in which various blood components sediment in order to further increase the efficiency of this apparatus.

HL-A matched platelets, autologous platelets from patients with leukemia in remission and pooled random donor platelets have been frozen using 5% DMSO and subsequently transfused to thrombocytopenic patients. Satisfactory clinical responses were obtained in about 35% of autologous platelet transfusions. Important technical factors appeared to be the concentration of platelets frozen and the type of plastic bag used. Presently, studies are underway utilizing polyolefin bags and lower platelet concentrations in order to improve upon these results.

In studies performed with Dr. R. Rubin, the rate of clearance of infused plasticizer (Di2-ethylhexylphthalate - DEHP) was measured in patients receiving platelet transfusions. $T\frac{1}{2}$ of the DEHP was 28 minutes and much of the infused DEHP was excreted in the urine within 24 hours. No toxicity was noted.

Investigations on the effects on platelet size and morphology of storage, cryopreservation, antiplatelet antibody and osmotic stress are in progress. Electron microscopy and a Coulter Channelyzer are being utilized in this project. Fixation of cells with 2.5% gluteraldehyde has been necessary to perform the ostmotic experiments.

A computer program was written which enables rapid selection of HL-A matched donors refractory to random donor platelet transfusions.

The ability of patients with leukemia receiving chemotherapy to produce lymphocytotoxic and ABO antibodies was analyzed.

II Granulocyte Transfusion - The mechanics of granulocyte collection have been well worked out and two satisfactory techniques, differential centrifugation, and filtration leukopheresis (FL) are available. There is an increased incidence of severe transfusion reactions following administration of cells obtained by FL. Preliminary work from our laboratory suggests that washing and incubating the cells in heparinized plasma may decrease the incidence of these reactions.

Hydroxyethyl starch (HES) can increase the yield of granulocytes obtained by differential centrifugation. The effect of HES on *in vitro* platelet and granulocyte function was measured.

Further investigations into the mechanism of neutropenia induced by FL were conducted.

I. Platelet transufsion - HL-A matched platelets have continued to produce good clinical results. Selection of donors using specific antiplatelet antibody testing (PF-3 release) has not been of value in our laboratory. Frozen platelets have produced mixed results. Freezing loss has been about 35% and is not related to freezing rate or length of storage.

HES does not affect platelet aggregation, size, nucleotide and PF-3 release, or morphology as viewed through phase microscopy. Preliminary ultrastructural studies demonstrated minimal surface changes in platelets exposed to HES.

Sixty-three per cent of patients with leukemia were able to produce ABO or lymphocytotoxic antibodies in response to platelet transfusions. Antibody production could not be correlated with prognosis, type or timing of chemotherapy, immunoglobulin levels, skin test reactivity or the number of transfusions received.

II Granulocyte Transfusions - Previous clinical impressions about the clinical efficacy of WBC transfusions in some patients were reconfirmed. HES was shown to have no effect on in vitro granulocyte function or morphology. Incubation of FL cells may decrease the frequency of transfusion reactions.

I Platelet Transfusion - The pool of HL-A matched platelet donors has been expanded and hopefully some patients will be able to receive matched platelets throughout their induction course. Measurement of platelet antibody by platelet aggregometry will be used in an attempt to select matched donors.

II Granulocyte Transfusion - The kinetics of neutropenia following FL will be studied using $DF^{32}P$. ^{99}TC will be used as a granulocyte label in order to perform scans in patients receiving granulocyte transfusions.

Keyword Descriptors: Platelet - Granulocyte transfusion, platelet cryopreservation, HES, DEHP, leukemia antibody

Honors and Awards: None

Publications:

1. Schiffer, C., Buchholz, D., Wiernik, P.: Intensive Multi-Unit Plateletpheresis of Normal Donors. Transfusion 14:388-394, 1974.

2. Schiffer, C., Buchholz, D., Aisner, J., Wiernik, P.: Clinical Experience with Transfusion of Granulocytes Obtained by Continuous Flow Filtration Leukopheresis. Am. J. Med. 58:373-81, 1975.

3. Schiffer, C., Aisner, J., Wiernik, P.: Transient Neutropenia Induced by Transfusion of Blood Exposed to Nylon Fiber Filters. Blood 45:141-46, 1975.

4. Schiffer, C., McCredie, K.: Cell Component Therapy for Patients with Cancer, Transfusion Therapy, American Association of Blood Banks, Washington, D.C., pp 29-54, 1974.

5. Schiffer, C., Buchholz, D., Aisner, J., Wiernik, P.: Clinical Experience with Transfusion of Granulocytes Obtained by Continuous Flow Filtration Leukopheresis. Proceedings of the International Symposium on Leucocyte Separation and Transfusion (in press).

6. Schiffer, C., Aisner, J., Wiernik,P.: Transient Neutropenia During Continuous Flow Filtration Leukopheresis. Proceedings of the International Symposium on Leucocyte Separation and Transfusion (in press).

7. Buchholz, D., Schiffer, C., Wiernik, P., Betts, S., Reilly, J.: Granulocyte Transfusion: Donor Response to Repeated Leukopheresis. Proceedings of the International Symposium on Leucocyte Separation and Transfusion (in press).

8. Buchholz, D., Schiffer, C., Wiernik, P., Betts, S.: Granulocyte Transfusion: A Low Cost Method for Filtration Leukopheresis. Proceedings of the International Symposium on Leucocyte Separation and Transfusion (in press).

9. Sanel, F., Aisner, J., Tillman, C., Schiffer, C., Wiernik, P.: Evaluation of Granulocytes Harvested by Filtration Leukopheresis: Functional, Histochemical and Ultrastructural Studies. Proceedings of the International Symposium on Leucocyte Separation and Transfusion (in press).

10. Buchholz, D., Schiffer, C., Wiernik, P., Betts, S., Reilly, J.: Granulocyte Transfusion: Donor Response to Repeated Leukopheresis. Transfusion, 1975.

11. Schiffer, C., Aisner, J., Schmukler, M., Whitaker, C., Wolff, J.: The Effect of Hydroxyethyl Starch on in vitro Platelet and Granulocyte Function. Transfusion, 1975.

Project No.: Z01 [illegible] 06276-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Chemotherapy of Metastatic Testicular Carcinoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Bruce Stechmiller, M.D.
John Wolff, M.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives are to assess the efficacy of various anti-tumor chemotherapeutic agents in metastatic testicular carcinoma. Patients are currently randomized between a combination of drugs consisting of fluorouracil, cyclophosphamide, methotrexate, and vincristine and a combination including the same drugs plus bleomycin given 12 hours after the dose of vincristine. Patients failing this protocol are randomized between adriamycin or IV methyl CCNU.

Twenty-one patients have currently been entered into the study. Objective tumor regression has been observed in 16 patients. Both regimens are generally well tolerated and no major life threatening toxicity has been encountered. It is too early to determine whether the addition of bleomycin to the other agents will result in a higher remission rate or longer remission duration.

Testicular carcinomas account for a significant proportion of tumors in young male adults. Metastatic testicular carcinoma has been shown to be responsive to several different classes of anti-tumor agents by this group and others. The present study shows promise of achieving results superior to those previously reported.

Project No.: Z01 CM 06276-02 BCRC

Keyword Descriptors: Chemotherapy, testicular carcinoma

Publications:

1. Sickles, E.A., Sklansky, B.C., and Wiernik, P.H.: Benign Intrathoracic Lesions Mimicking Recurrent Cancer. JAMA 225:156-158, 1973.

Project No.: Z01 CM 06277-04 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Chemotherapy of Adult Acute Leukemia

Previous Project Number: NCI-6277

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Michael J. O'Connell, M.D.
Charles A. Schiffer, M.D.
Paul Chang, M.D.
Sol Spiegelman, M.D.
Dwight Stickney, M.D.

Cooperating Units: Acute Leukemia Cooperative Group B
Research Microbiology, BCRC
Clinical Research Pharmacy Service, BCRC
Section of Biochemistry, Laboratory of Pharmacology, BCRC
Section of Neurological Surgery, BCRC
Francis Delafield Hospital, New York
Burroughs-Wellcome Company, North Carolina

Man Years:

Total:	5
Professional:	2
Other:	3

Project Description:

I. Effect of Single Agents

A. Daunorubicin:

Daunorubicin has been found in previous studies at this institution to be the most effective single agent in the treatment of adults with acute non-lymphocytic leukemia. Consequently, daunorubicin given in the dosage and schedule identified by our pharmokinetic studies with the Section of Biochemistry as the most efficacious was utilized as one arm of a controlled Phase III study in adult acute non-lymphocytic leukemia. To date 33 previously untreated patients have been entered on-study, and all are evaluable at the time of this report. The complete remission rate with this drug alone is 48% of all patients entered on protocol. If all patients over the age of 70 are omitted from evaluation, the complete remission rate is 52%. The median survival of patients treated with daunorubicin alone as of the date of this report is approximately 11 months. The median survival of all patients who achieved a complete response is 14 months. These data confirm and extend our previous observations with daunorubicin.

B. Azacytidine vs. Guanazole:
This is a Phase II study designed to determine the relative efficacy of two agents recently demonstrated to have activity in acute leukemia, namely azacytidine and guanazole. There are 18 evaluable previously treated patients on the azacytidine arm, 5 of which have achieved a complete remission. Only one partial remission was seen in 12 evaluable patients treated with guanazole. Azacytidine will be investigated further earlier in the therapy of this disease.

II. Effect of Multiple Drugs in Combination

A. Daunorubicin, Cytosine Arabinoside, Thioguanine and Pyrimethamine:
This program forms the second randomization in our current protocol for adult acute non-lymphocytic leukemia. Pyrimethamine is included in this combination of agents in an attempt to treat occult central nervous system leukemia at the time of diagnosis with an agent that can be given orally. Researchers at Burroughs-Wellcome and Company, Research Triangle, North Carolina have measured pyrimethamine levels in cerebral spinal fluid from our patients treated on this arm of the study and have identified the compound in adequate concentration for prolonged periods of time. Thirty-three previously untreated patients have been entered on this arm of the protocol, and all are evaluable at the time of this report. The complete remission rate for all entered patients is 45%. If all patients over the age of 70 are excluded from evaluation, the complete remission rate is 50%. The median survival of patients who achieved a complete response is 13 months. There is evidence from this study that the administration of pyrimethamine at the time of the initial therapy in adult patients with acute non-lymphocytic leukemia is useful in delaying the onset of meningeal leukemia.

B. Daunorubicin, Cytosine Arabinoside and Thioguanine; Cytosine Arabinoside and Thioguanine; and Daunorubicin Alone:
This study is being chaired by the principal investigator for Acute Leukemia Group B (ALGB #7221). As of the time of this report, 523 patients have been entered and 460 are evaluable. The remission rate is over 50% in each arm of the study and the median duration of complete remission is approximately 6 months in all 3 arms.

C. Vincristine, Thioguanine, Pyrimethamine and Dexamethasone vs. Vincristine, Thioguanine, Methotrexate and Prednisone for Remission Induction in Adults with Acute Lymphocytic Leukemia:
This program is designed to test whether or not pyrimethamine administered initially to adults with acute lymphocytic leukemia can prevent or delay the onset of meningeal leukemia in long-term survivors.

Additionally, the protocol was designed to test whether or not dexamethasone, a steroid that mobilizes granulocytes might be associated with fewer infections during induction than prednisone, a steroid that inhibits granulocyte mobilization. A complete remission was obtained in 53% of patients in the median duration of survival of remitting patients is in excess of 18 months. Approximately 40% of patients on the study have developed meningeal leukemia.

D. Azacytidine and Methylglyoxal Bis (Guanylhydrazone) in Previously Treated Adults with Acute Nonlymphocytic Leukemia:
Eight patients have been treated with this combination of agents and thus far, only one partial response has been seen. Because of the severe toxicity of this combination, the study has been closed.

E. Vinblastine, Azacytidine, and VP16-213 for the Treatment of Relapsed Patients with Acute Nonlymphocytic Leukemia:
Four patients have been entered onto this study and one has obtained a complete remission. It is too early to evaluate the study further and it will continue.

F. Cytosine Arabinoside and Daunorubicin in Intensive Prolonged Courses for the Treatment of Newly Diagnosed Patients with Acute Nonlymphocytic Leukemia:
To date 21 patients have been entered on this study and 16 (76%) have achieved complete remission. These are the best complete remission rates obtained in the therapy of this disease in this institution and are among the best reported in the literature. It is too early to evaluate this study further and patient accrual will continue.

III. Maintenance Therapy for Remission in Adults with Acute Non-lymphocytic Leukemia.

A. Cytoxan and Guanazole:
All patients achieving complete remission on the daunorubicin alone arm or the daunorubicin, cytosine arabinoside, thioguanine and pyrimethamine arm of our protocol #7110 are maintained with monthly injections of cytoxan 1500 mg/M^2 in a 24 hour I.V. infusion. It is hoped that cells in G_0 phase might be recruited into the cell cycle by the agent cytoxan. Subsequent treatment with guanazole, an S-phase active agent, might then accomplish a greater degree of cell kill than if used alone. The combination of these agents used in this way might thereby prolong the duration of remission over that which is customarily achieved in this disease. At present the median duration of first remission in both arms of the study is approximately 7 months. This figure compares favorably with the 2.3 months duration of unmaintained remission seen in a previous study here utilizing daunorubicin or POMP.

IV. Biochemical Indicators of Disease Activity in Acute Non-lymphocytic Leukemia

A. Serum and Urine Muramidase Studies:

We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen:

We have observed that approximately 30% of adult acute non-lymphocytic leukemia or acute lymphocytic leukemia have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse, the presence or absence of CEA in their plasma at that time does not correlate with findings at the time of presentation.

C. Serum Immunoglobulin Concentrations in Patients with Acute Non-lymphocytic Leukemia:

We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved, these levels become normal.

D. Reverse Transcriptase in Leukemic Cells:

This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point. 40 patients have been studied prior to therapy and the enzyme was present in the white cells of each. In addition, most patients in complete remission continue to have the enzyme detectable in their white cells.

E. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia:

Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 98 patients by recall-antigen skin testing and in 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis, in terms of attainment of complete remission of his leukemia, duration of remission, and survival could not be correlated with the pretherapy demonstration of intact delayed cutaneous hypersensitivity.

Summary of Major Findings:

1. Daunorubicin and cytosine arabinoside given in intensive courses produce a complete remission rate superior to that of other regimens tested at this institution.
2. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.
3. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. However, serial determinations are useless in following disease activity.
4. Cell-mediated immunity in leukemia patients prior to therapy may not be important in determining response.
5. The presence of reverse transcriptase in the white cells of leukemia patients in remission may indicate the necessity for further induction therapy.

Thse studies indicate continued improvement in our ability to arrest disease activity in adults with acute non-lymphocytic leukemia in an ever increasing number of patients. In addition, biochemical parameters are being identified that reflect accurately disease activity in many patients with acute non-lymphocytic leukemia. The identification of such factors may lead to more rational therapy in the future. Demonstration of reverse transcriptase in leukemic cells may have etiologic significance.

Keyword Descriptors: Acute leukemia, chemotherapy

Publications:

1. Rosenoff, S.H., Canellos, G.P., O'Connell, M. and Wiernik, P.H.: Mediastinal Adenopathy in Granulocytic Leukemia. Arch. Int. Med.,134:135-138, 1974.
2. Wallace, H.J., Holland, J.F., Glidewell, O.J., Ellison, R.R., Weil, M., Carey, R.W., Schwartz, J.M., Hoagland, H.C., Henderson, E.S., Wiernik, P., Yates, J.W.: Therapy of Acute Myelocytic Leukemia - Acute Leukemia Group B Studies. Minerva Med., in press, 1975.
3. Schiffer, C.A., Sanel F.T., Stechmiller, B.K., Wiernik, P.H.: Functional and Morphologic Characteristics of the Leukemic Cells of the Patient with Acute Monocytic Leukemia: Correlation with Clinical Features. Blood, in press, 1975.
4. Wiernik, P.H., Schimpff, S.C.: Daunorubicin Alone Vs. Daunorubicin, Pyrimethamine, Cytosine Arabinoside, and Thioguanine for the Treatment of Acute Non-Lymphocytic Leukemia. Proc. Amer. Soc. Clin. Oncol., in press, 1975.
5. Smyth, A.C., Wiernik, P.H., Serpick, A.A.: Therapy of Adult Acute Lymphocytic Leukemia with Thioguanine, Oncovin, Daraprim, and Dexamethazone: Proc. Amer. Soc. Clin. Oncol., in press, 1975.
6. Wiernik, PH., Glidewell O., Holland J.F.: Comparison of Daunorubicin with Cytosine Arabinoside and Thioguanine and with a Combination of All Three Drugs for Induction Therapy of Previously Untreated AML. Proc. Amer. Assn. for Cancer Res., in press, 1975.

7. Viola, M.V., Frazier, M., Baxt, W.G., Wiernik, P., Gee, T.: Leukemia - Specific DNA Sequences in "Normal" Leukocytes of Leukemia Patients in Remission. *XI International Cancer Congress*, Vol. 1, p. 253, 1974.

8. Levi, J.A., Wiernik, P.H.: A Comparative Clinical Trial of 5-Azacytidine and Guanazole in Previously Treated Adults with Acute Non-Lymphocytic Leukemia. *Cancer*, in press, 1975.

Project No.: Z01 CM 06280-04 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Combination Chemotherapy for Stage IIIB and IV Hodgkin's Disease

Previous Project No.: Same

Principal Investigators: Peter H. Wiernik, M.D., John A. Levi, M.D.

Other Investigators: Larry Kvols, M.D.

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine whether a combination of cyclophosphamide, vinblastine, procarbazine and prednisone might prove efficacious as an induction regimen in the therapy of advanced Hodgkin's disease. To determine if maintenance therapy with CCNU and vinblastine prolongs remission duration and/or survival.

Fifty-one patients with advanced Hodgkin's disease have been entered on this study. Forty-five have completed induction therapy and are considered evaluable. Sixteen of the patients had Stage III disease and 20 had Stage IV disease. Among the 19 previously untreated patients, 13 (68%) achieved a complete remission. Fourteen of the 17 patients previously treated with major radiotherapy achieved complete remission (82%). Nine of the patients had previously been treated with both major radiotherapy and chemotherapy and only four of these (44%) achieved a complete remission.

Myelosuppression was the major toxicity with 20 of 45 patients having white cell counts of 1000 per cubic millimeter or less during one or more of the cycles. The leukopenia was of short duration and there was only one leukopenic related death from induction therapy. Thrombocytopenia was less common and only 6 of 45 patients had platelet counts less than 50,000 per cubic millimeter. Six episodes of hemorrhagic cystitis were seen and one patient developed vinblastine neurotoxicity.

Of the 29 patients in complete remission who have been randomized on the maintenance regimen, 17 have or presently are receiving maintenance therapy and 12 received no maintenance. As few patients have relapsed on either arm of this study to date, it is too early to determine whether or not maintenance will have a beneficial effect on remission duration and perhaps survival. In conclusion, CVPP is an effective induction regimen for advanced Hodgkin's disease, including those patients with prior therapy. Maintenance therapy may have a beneficial effect on survival.

Although many combinations of effective agents have been identified for the treatment of advanced Hodgkin's disease, in the best hands 20% of patients fail to respond significantly. If this study will result in a regimen that produces a longer duration of response than obtained with other regimens, the therapy of disseminated Hodgkin's disease will have been improved.

Continue present Study.

Keyword Descriptors: Hodgkin's disease, chemotherapy

Honors and Awards: None

Publications:

1. Kvols, L., Diggs, C., Levi, J.: CVPP (Cyclophosphamide, Vinblastine, Procarbazine, Prednisone) for Advanced Hodgkin's Disease: Efficacy of Maintenance Therapy. Proceedings of the AACR (in press).

Project No.: ZO1 CM 06293-04 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Teaching and Consultation Service in Medical Oncology

Previous Serial Number: NCI-6293

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert Esterhay, M.D.
Stephen Schimpff, M.D.
Michael J. O'Connell, M.D.
Charles Schiffer, M.D.
John Levi, M.D.

Cooperating Units: University of Maryland School of Medicine
Johns Hopkins University School of Medicine
Greater Baltimore Medical Center
Maryland General Hospital
Bon Secours Hospital

Man Years:
Total: 1 1/21 1/2
Professional: 1 1/2
Other: 0

Project Description:

The objective is to teach the theory and practice of medical oncology to medical students and house officers. To acquaint such individuals with newer aspects of chemotherapy, radiotherapy and surgery as they apply to neoplastic diseases. To provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area free of charge. To teach basics of cancer nursing to physician assistant students.

During their second year, medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition,house officers from the Greater Baltimore Medical Center and Maryland General Hospital have been assigned here for elective periods of their house officer year. University of Maryland Assistant Residents in Medicine rotate through the Section of Medical Oncology on a regular basis. Physicians in the community refer patients here for an out-patient consultation. If the patient meets the qualifications for one of our protocol studies, he is accepted in transfer and admitted to BCRC.

If not, the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy.

Students, house officers, and physician assistants who have rotated through the BCRC are recognizing that medical oncology is a bona fide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care in order to achieve good results. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at this time, such as early during their student years and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

Keyword Descriptors: Cancer Education

Honors and Awards: None

Publications: None

Project No.: Z0[illegible] 06295-04 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Census: A Remote Access Patient Information Storage and Retrieval System.

Previous Serial Number: Same

Principal Investigators: William L. Robinson
Robert J. Esterhay, M.D.

Other Investigators: Paul Chang, M.D.

Cooperating Units: Section of Neurological Surgery, NCI, BCRC
Nursing Service, NCI, BCRC
Section of Microbiology, NCI, BCRC
Clinical Research Pharmacy Service, NCI, BCRC
Adminstrative Officer, NCI, BCRC
Section of Medical Oncology, NCI, BCRC

Man Years:		
	Total:	3
	Professional:	3
	Other:	0

Project Description:

Objectives:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible, and maintained by a single non-technical individual.
2. To generate a daily report on all in-patients and their locations, along with their vital statistics and current therapy data, to be distributed to all involved in the tasks of clinical research, patient care and administration.
3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

Methods Employed:

1. Information is compiled and recorded daily in a data input log by a medical secretary.
2. A computer program accepts the input datum from the secretary, checks for logical content and accuracy, and records it on the data base.
3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.
4. The report is then distributed to the scientific and professional staff, remote contracting laboratories, and administrative office.
5. The programs and data base are executed and stored on a commercial time-sharing service.

6. Mass memory disk files and remote terminals form the storage and access mediums.

The daily census report has been utilized by all sections and services of the Cancer Center.

1. Admissions Officer: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.
2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.
3. Sections of Medical Oncology and Neurological Surgery: the master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.
4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.
5. Section of Microbiology: provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRC (see Project Report Z01 CM 06039-03 BCRC).
6. A general-purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.
7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Four years of continuous operation of the Census System have provided BCRC with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by other data systems at the BCRC.

Keyword Descriptors: patient, information, storage, retrieval, data base, computer, census, cancer, patients

Honors and Awards: None

Publications: None

Project No.: Z01-CM 06299-03 BCRC
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Therapy of Patients with Metastatic Sarcoma

Previous Serial Number: SAME

Principal Investigators: Paul Chang, M.D.
Peter H. Wiernik, M.D.

Other Investigators: Stephen Schimpff, M.D., Nicholas R. Bachur, M.D., Ph.D.

Cooperating Units: Biochemistry Section, BCRC

Man Years:

Total:	½
Professional:	½
Other:	0

Project Description:

A) Adriamycin vs. Adriamycin + Streptozotocin for Previously Untreated Patients with Metastatic Sarcoma - Although uncommon in adults, soft tissue and bone sarcomas constitute a major group of fatal malignancies in children. Until recently, Ewing's sarcoma and childhood embryonal rhabdomysarcoma were the only tumors of this group which were more than occasionally responsive to chemotherapy. Recent studies at BCRC and elsewhere have demonstrated significant, clinically useful responses in 25-40% of patients treated with adriamycin with mild marrow and minimal mucosal toxicity. Phase II studies at this center have also found anti-sarcoma activity with streptozotocin, a non-myelosuppressive agent. Therefore, a randomized clinical trial has been undertaken comparing the efficacy of adriamycin alone to adriamycin + streptozotocin given simultaneously in previously untreated patients with inoperable or metastatic sarcomas without evidence of central nervous system spread. Patients who have or develop CNS metastases will be treated according to part B of this project

Adriamycin vs. Adriamycin + Streptozotocin - Twenty-four evaluable patients have now received adriamycin (14 pts.) or adriamycin + streptozotocin. Demographic characteristics and median performances status are similar in the two groups. Responses (at least stabilization of previously advancing disease) have been achieved in 8/14 patients on adriamycin and 7/10 patients on the combination with partial remissions (> 50% tumor shrinkage for 1 month with subjective improvement) in 4/14 and 2/10 pts. in each treatment group, respectively. It is still too early to discern a difference in remission duration and survival.

B) Intravenous Methyl CCNU for Advanced Sarcoma - The Phase I study of methyl CCNU administered intravenously (I.V. methyl CCNU) at the BCRC last year demonstrated stabilization or regression of tumor in three of seven previously extensively treated patients with metastatic sarcoma. All patients were previously extensively treated patients with metastatic sarcoma. All patients were previously shown to be refractory to adriamycin. Pharmacokinetic studies of this highly lipid-soluble agent demonstrated a significant penetration of drug radio-labelled C^{14} into CSF. Thus, a Phase II study with I.V. methyl CCNU has been started in patients with metastatic sarcomas refractory to adriamycin and in patients with central nervous system metastases from sarcomas.

Intravenous Methyl CCNU for Advanced Sarcomas - Twenty eight patients have received I.V. methyl CCNU on this study. Seven have had at least stabilization of disease: 2 patients (one with myxoid chondrosarcoma and one with embryonal rhabdomyosarcoma) have had > 50% tumor decrease, 3 pts. (1 each with alveolar rhabdomyosarcoma, malignant giant cell tumor of bone, and malignant fibrous histiocytoma) have had <50% tumor decrease, and 2 pts. with leiomyosarcoma had stabilization of previous advancing disease. Onset of response was quite prompt, occurring 4-9 days after the drug was begun. Clinically significant benefit to the patients occurred only in the 2 pts. who achieved partial remissions. Both of these patients achieved pain-free, fully ambulatory status, having been bedridden in one case and in a wheelchair in the other prior to this drug treatment.

In conclusion, methyl CCNU given intravenously has only a low degree of anti-sarcoma activity. It is felt that the low degree of activity does not warrant Phase III evaluation in sarcomas. Furthermore, the low activity plus significant delayed myelosuppression argue against inclusion of this agent into combination for treating sarcomas. This study has been terminated.

C) Adriamycin + MER for Advanced Sarcoma - As indicated in A) above, adriamycin has now become the cornerstone of therapy of inoperable or metastatic sarcomas having been identified as the most effective single drug in this group of tumors. It has, however, significant dose-limiting toxicity, in that 10-30% of patients who receive > 500 mg/m^2 (cumulative dose) later suffer irreversible cardiac failure.

MER, the methanol-extraction residue of BCG tubercle bacilli, is thought to be a non-specific stimulant of the immune response with some anti-tumor properties in animal tumor models. The drug may also be able to decrease the myelosuppression seen with cytotoxic agents. Although no patients with sarcoma have been treated, a Phase II trial of MER alone in patients with colon cancer demonstrated minimal systemic toxicity and partial remissions were seen in 2 of 34 patients and a complete remission seen in 1 patient treated with this drug. A pilot study has therefore been started to evaluate the combination of adriamycin and MER in patients with advanced sarcomas to attempt to both increase the rate of remission and decrease the myelosuppression, as well as prolong the duration of remissions.

This study has only recently been initiated and insufficient data is available for discussion.

Project No.: Z01 CM 06299-03 BCRC

Keyword Descriptors: Sarcoma, chemotherapy.

Honors and Awards: None

Publications: None

Project No.: Z01-CM 06913-03 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer

Previous Serial Numbers: Same

Principal Investigator: Joseph Aisner, M.D.

Other Investigators: Larry K. Kvols, M.D., Stephen C. Schimpff, M.D., Viola M. Young, Ph.D., Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, BCRC, and Pathology and Radiology Departments, University of Maryland Hospital

Man Years:

Total:	½
Professional:	½
Other:	0

Project Description:

The cancer patient is frequently immunologically compromised and myelosuppressed. Such patients often develop pulmonary complications, especially infection. Such pulmonary infections are a contributing factor in the demise of approximately half of these patients. A major reason for the high mortality lies in difficulty in establishing an etiologic agent and thereby providing appropriate antimicrobial therapy. Several investigators have emphasized the low yield of noninvasive culture techniques in pneumonia occurring in patients with hematological malignancies. Furthermore, myelosuppressed patients frequently carry gram negative organisms and fungi in their nasooropharynx which will contaminate expectorated sputum. The purpose of this study is to establish a method for diangosing unexplained pulmonary infiltrates where conventional methods (sputum induction and transtracheal aspiration) have been unseccussful. This study utilized a transtracheal approach with selective bronchial brushing, and percutaneous lung biopsy when other methods have failed.

As of February, 1975, 37 patients with diffuse or nodular pulmonary infiltrates have undergone transtracheal bronchial brushing. Conventional methods, including transtracheal aspiration, failed to establish a diagnosis in all patients. Eleven of the 33 patients were on broad spectrum antibiotics for fever, complicating subsequent interpretation. Eleven of 14 patients subsequently proven to have infection were diagnosed by brushing. One additional patient was shown to have metastatic sarcoma. Diagnosis established by the brushing procedure included: Aspergillus pneumonia, Phycomycetes pneumonia, T. glabrata pneumonia, Staphylococcal pneumonia, Streptococcal pneumonia, anaerobic bacterial pneumonia, and metastatic sarcoma.

There was no mortality associated with the procedure. Morbidity was acceptable including four cases of pneumothorax and one case of mediastinal emphysema, and one case of severe cellulitis at the tracheal puncture site. Despite pancytopenia, bleeding was not a problem.

The proposed course is to continue investigation.

Keyword Descriptors:

Bronchial brushing, pulmonary infiltrates, pneumonia, fungal pneumonia, infection.

Honors and Awards:

None

Publications:

1. Sickles EA, Young VM, Greene WH, Wiernik PH: Pneumonia in acute leukemia. Ann Intern Med 79:528-534, 1973.

2. Aisner J, Sickles EA, Schimpff SC, Young VM, Greene WH, Wiernik PH: Torulopsis glabrata pneumonitis in patients with cancer. JAMA 230:584-585, 1974.

3. Aisner J, Schimpff SC, Bennett JE, Young VM, Wiernik PH: Aspergillus infections in patients with cancer: An association with fire-proofing materials in a new hospital. JAMA (in press).

4. Aisner J, Schimpff SC, Bennett JE, Sutherland JC, Young VM, Wiernik PH: Increased incidence of Aspergillus infections in cancer patients: An association with fire-proofing materials. Clin Res (in press).

5. Aisner J, Schimpff SC, Sutherland JC, Young VM, Wiernik PH: Torulopsis glabrata infections in patients with cancer. Clin Res (in press).

Project No.: Z01 CM 06914-02 BCRC
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intensive Supportive Care Alone Versus Intensive Supportive Care Plus Radiotherapy for Localized Inflammatory Lesions in Patients with Acute Leukemia

Previous Project No.: Same

Principal Investigator: John A. Levi, M.D.

Other Investigators: Stephen C. Schimpff, M.D., Peter H. Wiernik, M.D.

Cooperating Units: Section of Radiotherapy, University of Maryland Hospital

Cooperating Investigators: Robert A. Slawson, M.D.

Man Years:
Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To define the optimal management of these localized lesions in patients with acute leukemia and in particular to determine in a controlled fashion whether low-dose irradiation therapy administered to sites of local inflammation and infection will facilitate the healing process and prevent the development of disseminated infection with its associated morbidity and mortality.

Localized irradiation therapy has been utilized in the past in uncontrolled studies in patients with leukemia and results achieved have suggested some benefits to be derived from its use.

Patients with acute leukemia who develop a localized superficial inflammatory lesion which is not secondary to a previously proven disseminated infection are randomized to receive local care alone to these lesions or local care plus 400 rads of megavoltage irradiation directed to the local lesion and given over a 2 day period. A further 400 rads is given in 7 days irrespective of immediate results. Patients are separated into three groups: a) perianal or perineal lesions, b) axillary lesions, c) other sites. All patients go down to the Radiotherapy Department and allocations to radiotherapy are not communicated to the clinical staff until the patients trial has ended.

Twenty-two patients have been entered into this study, eleven patients have received local radiotherapy and all lesions completely regressed by day 11. Eleven patients did not receive radiotherapy and eight had complete resolution of lesions by day 16, one partial resolution after 3 weeks and two lesions progressed despite local care. One patient in the nonirradiated group has suffered from disseminated infection as a result of the local lesions. There have been nine recurrences of these local lesions amongst a total of 22 patients, and three of these recurrences have occurred in sites previously irradiated while six recurred in sites that were not previously irradiated. Although it is not possible as yet to come to any meaningful conclusions. it would appear that localized radiotherapy has had some beneficial effects on the rate of resolution and prevention of recurrences. However, it is not apparent that the addition of irradiation to local therapy has been of any particular value in preventing disseminated infection.

Overwhelming infection remains the principal cause of death in patients with acute leukemia and further methods to prevent and treat such infections are required. Patients with acute leukemia have reduced body defenses to cope with local infections and a significant mortality has been associated with dissemination from these sites in the past. The early results reported here show some promise for the use of local radiotherapy in speeding the healing of such lesions and preventing recurrences. However, all forms of supportive care appear able to prevent disseminated infection.

Keyword Descriptors: Infection, acute leukemia

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06915-02 BCRC
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Staging Laparotomy and Combined Modality Therapy in Stage IA and IIA Lymphocyte Predominant, Nodular Sclerosing, and Mixed Cellularity Hodgkin's Disease

Previous Project No.: Same

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: Robert A. Slawson, M.D.

Cooperating Units: Radiotherapy Service and Surgery Service, University of Maryland Hospital

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

1. To determine whether modification of radiation therapy ports on the basis of staging laparotomy findings will significantly improve survival in patients with clinically localized Hodgkin's disease, as compared to radiation therapy administered without staging laparotomy.

2. To determine whether the addition of adjuvant chemotherapy to limited field radiation therapy will result in improved survival compared to limited field radiation alone or extended field radiation alone in patients with clinically localized disease who have undergone staging laparotomy.

Patients with newly diagnosed Hodgkin's disease undergo complete clinical evaluation including history, physical examination, serum chemistries, and lymphangiography. At this point patients are randomized to receive extended field radiation therapy alone, or alternatively to undergo staging laparotomy with subsequent therapy modified according to findings at surgery. Patients who undergo laparotomy are randomized to receive one of the 3 following regimens: 1) limited field radiation therapy alone, 2) extended field radiation alone, or 3) limited field radiation therapy followed by chemotherapy using nitrogen mustard, vincristine, prednisone and procarbazine.

Eighteen patients have been entered into this study up to this point, three patients were randomized to receive no laparotomy and extended field radiation alone. All three obtained a complete remission and this persists to the present time (range 1-12 months). The remaining 15 patients all had laparotomies and were pathologically staged as I-IIA disease. Further randomization resulted in one patient having received involved field irradiation alone, eight patients extended field irradiation alone and six patients involved field irradiation plus MOPP chemotherapy. There have only been two patients who have relapsed up to this time and both had received extended field irradiation alone, relapses occurred at three and eight months following the completion of therapy. It is too early at this time to make any assessment of the relative value of the various approaches outlined in this protocol.

Although staging laparotomy has been demonstrated to be of considerable benefit to many patients with Hodgkin's disease, there still exists controversy as to its value in patients with early localized Hodgkin's disease. This study should allow direct comparison of complication rates and overall survival in patients treated with radiation alone who have undergone staging laparotomy as opposed to patients treated with radiation alone who have not undergone staging laparotomy in a prospectively randomized clinical trial. In addition, the possible benefits of adjuvant chemotherapy and/or extended field radiation as opposed to involved field radiation should be evaluable, and may allow selection of the most effective and least toxic treatment regimen for patients with this potentially curable disorder.

Keyword Descriptors: Hodgkin's disease, laparotomy

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06916-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pilot Protocol for the Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma

Previous Project No.: Same

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: Stephen Schimpff, M.D., Larry Kvols, M.D.,
Charles Schiffer

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine whether high dose intensive combination chemotherapy of patients with diffuse histiocytic lymphomas, utilizing measures to prevent infection such as laminar air flow isolation units and prophylactic oral nonabsorbable antibiotics, will result in an improved remission rate and a prolonged survival.

Patients with biopsy proven malignant lymphoma of the diffuse histiocytic variety who have clinical stage III or stage IV disease are placed in reverse isolation in a laminar air flow unit, if available, and given prophylactic nonabsorbable oral antibiotics. These patients have been treated with doses of chemotherapeutic agents designed to produce significant myelosuppression in an attempt to provide better control of this devastating disease than is possible with conventional doses of the same drugs. The agents employed in this study are cytoxan, adriamycin, vincristine and prednisone.

Three patients have been entered on the study to date. Excellent tolerance to the high doses of chemotherapeutic agents employed, the stress of reverse isolation, and the administration of the oral antibiotics was observed in these patients. Two patients have completed induction and both achieved complete remission which continue (3 and 16 months). The third patient has shown marked regression of disease after three courses of chemotherapy. Further patients are needed to more adequately evaluate this approach.

Disseminated diffuse histiocytic lymphoma is the most malignant variety of non-Hodgkin's lymphomas, with a median survival of less than one year from the time of diagnosis. Conventional chemotherapeutic regimens, although frequently producing some disease regression, are commonly ineffective in producing lasting disease control. Thus, if the infectious complications which are associated with severe bone marrow depression from chemotherapy can be avoided by the use of laminar air-flow units and prophylactic oral nonabsorbable antibiotics, it may be possible to administer much higher doses of chemotherapy and thereby effect a more useful and long lasting remission of the disease.

Keyword Descriptors: Histiocytic lymphoma

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06917-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Cyclophosphamide, Adriamycin, Vincristine and Prednisone vs. Cyclophosphamide, Vincristine, and Prednisone for Disseminated Non-Hodgkin's Lymphoma

Previous Project Number: Same

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: Larry Kvols, M.D., Charles Schiffer, M.D., Stephen Schimpff, M.D.

Cooperating Units: None

Man Years:

Total: 1.0
Professional: 1.0
Other: 0

Project Description:

1. To determine whether the addition of adriamycin to cyclophosphamide, vincristine, and prednisone will improve remission induction rates in patients with disseminated non-Hodgkin's lumphomas.
2. To evaluate the efficacy of maintenance chemotherapy in prolonging both remission duration and survival in those patients who achieve complete remission following induction therapy.

Patients who are found to have stage III or stage IV non-Hodgkin's lymphomas on the basis of clinical staging procedures are randomly assigned to receive therapy with either cyclophosphamide, 1000 mg/m^2 on day 1 IV, adriamycin, 45 mg/m^2 on day 1 IV, vincristine 1.4 mg/m^2 on days 1 and 8 IV and prednisone 40 mg/m^2 on days 1 to 10 orally or cyclophosphamide 1500 mg/m^2 on day 1 IV, vincristine 1.4 mg/m^2 IV on days 1 and 8 and prednisone 40 mg/m^2 on days 1 to 10 orally without adriamycin for induction therapy. Patients who achieve complete remission then undergo consolidation therapy with CCNU, bleomycin and prednisone. A second randomization is then carried out with half the patients assigned to no further therapy and the remainder randomized to receive intermittent high dose cytoxan monthly intravenously until relapse occurs.

Sixteen patients have been entered to date, nine receiving therapy with CAVP and seven patients receiving CVP. Three of the patients receiving

CAVP are too early for evaluation and among the remaining six patients, five complete remissions have been achieved while one patient failed induction therapy. Of the seven patients receiving CVP therapy, five are evaluable and four complete remissions and one partial remission was achieved. It is too early to assess the results of remission duration of this group of patients.

Conventional chemotherapy regimens have produced at best a 50% complete response rate in the non-Hodgkin's lymphomas, with the majority of patients ultimately relapsing and dying of progressive disease from complications of its therapy. Furthermore, an attempt to provide curative therapy, modeled after the approach which has been successful in acute lymphatic leukemia, employing intensive consolidation therapy is being studied. This study is designed to determine whether these results can be improved by adding the new anthracycline antibiotic adriamycin to the induction therapy. Finally, it may be possible to determine the need for long term maintenance therapy in patients who achieve remission through the randomized scheme employed in this study.

Keyword Descriptors: Non-Hodgkin's lymphoma, chemotherapy

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06918-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Streptozotocin, Bleomycin, CCNU and Adriamycin for the Treatment of Advanced, Refractory Hodgkin's Disease

Previous Project No.: Same

Principal Investigator: John A. Levi, M.D.

Other Investigators: Peter H. Wiernik, M.D., Larry Kvols, M.D.

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine the clinical usefulness of a new combination chemotherapy regimen for advanced Hodgkin's disease which is no longer responsive to conventional chemotherapy approaches.

Patients with advanced biopsy proven Hodgkin's disease who have failed conventional chemotherapy approaches are treated with a combination of streptozotocin 0.5 gm/m^2 daily for 5 days, CCNU 100 mg/m^2 on day 1, bleomycin 15 mg/m^2 intramuscularly on days 1 and 8, and adriamycin 45 mg/m^2 on day 1 with cycles repeated each 28 days if blood counts permit.

Ten patients have been currently entered onto this study. Three are too early for evaluation and amongst the remaining 7 patients there have been 3 complete remissions, 1 partial remission and 3 failures, a remission rate of greater than 50%. The duration of the unmaintained complete remissions have been 3, 6, and 9 months. This combination of agents appears to have definite efficacy in this previously treated group of patients with Hodgkin's disease but it is too early as yet to draw firm conclusions.

Although currently available combination chemotherapy regimens, such as MOPP, have been shown to produce complete remissions in approximately 3/4 of patients treated, there is still a sizable number of patients with previously untreated Hodgkin's disease who do not achieve remission utilizing these drugs. In addition, patients treated with radiation therapy for local disease occasionally relapse and a proportion of these patients prove refractory to standard chemotherapy approaches. Therefore, a need for an effective alternative regimen exists. Streptozotocin has been shown in preliminary stage II studies to have definite activity against refractory Hodgkin's disease and is an attractive agent for combination chemotherapy since it has very mild bone marrow suppressive characteristics. Adriamycin, likewise, is a new agent which has been shown to have activity against Hodgkin's disease whose structure and mechanism of action is quite different than the agents commonly employed. Bleomycin has produced objective regressions in otherwise resistant Hodgkin's disease and it too has little bone marrow supressive characteristics. CCNU has been shown to have significant activity in patients refractory to alkylating agents and vinca alkaloids. Thus, it is hoped that the combination of these four different chemotherapeutic agents with different mechanisms of action and different toxicities will be of benefit to patients with HOdgkin's disease who are not responsive to standard chemotherapy regimens.

Keyword Descriptors: Hodgkin's disease, chemotherapy

Honors and Awards: None

Publications: None

Project No.: Z01-CM 06919-01 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Initial Drug Therapy for Metastatic Renal Cell Carcinoma

Previous Serial Number: Same

Principal Investigator: John C. Ruckdeschel, M.D.

Other Investigators: Peter H. Wiernik, M.D., Stephen C. Schimpff, M.D.

Cooperating Units: Department of Hematology, Veterans Administration Hospital, Seattle, Washington (Dr. R. Adamson)

Man Years:

Total: ½
Professional: ½
Other: 0

Project Description:

Objectives:

Renal cell carcinoma is a solid tumor of notorious resistance to chemotherapy and radiation therapy. Although surgery may be curative in some cases, the overwhelming percentage of cases proceed to metastatic disease and death within two years of diagnosis. Previous chemotherapy studies have been, almost without exception, inadequate. The few studies that have had adequate patients and adequate controls have indicated either no activity for such drugs as adriamycin or minimal activity for such drugs as provera. One agent that has received some notice in terms of treatment of renal cell carcinoma in CCNU; a response rate on the order of 10-15% has been seen in one study with this agent, and a similar response rate for vinblastine was noted in another study. Dr. James Holland recently reviewed material on a derivative of thio-TEPA that underwent evaluation in the Soviet Union for use in renal cell carcinoma and exhibited a response rate on the order of 50%. Previous work in this country with thio-TEPA was limited to a few case reports a number of years ago and the drug has never been thoroughly evaluated here. The major objective of this study will be to examine the efficacy of vinblastine, CCNU, or thio-TEPA in standard doses for the initial management of metastatic renal cell carcinoma.

Major Findings:

None to date.

Keyword Descriptors:

Renal carcinoma, chemotherapy

Honors and Awards:

None

Publications:

None

Project No.: Z01 CM 06920-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: 5-Azacytidine vs. Guanazole for the Therapy of Previously Treated Adult Acute Non-Lymphocytic Leukemia

Previous Project No.: Same

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik,M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine the relative efficacy of two new experimental chemotherapeutic agents, 5-azacytidine and guanazole, in the treatment in advanced adult acute non-lymphocytic leukemia which is no longer responsive to conventional chemotherapeutic agents.

Thirty patients were entered into this study. Eighteen received 5-azacyticine and 6 achieved remission, 5 of which were complete (28%). The median duration of complete remission was 100 days. Twelve patients received therapy with guanazole and only one acheived partial remission. Principal toxicities encountered with the 5-azacytidine therapy included gastro-intestinal intolerance, fever and neuromuscular toxicity. Fever was the principal toxicity observed after guanazole therapy, one patient developed erythema nodosum and another recurrent pulmonary infiltrate following guanazole therapy. 5-azacytidine therefore has significant activity as an induction agent in ANLL but guanazole appears to have no benefit for these patients.

Keyword Descriptors: acute leukemia, chemotherapy

Honors and Awards: None

Publications: None.

Project No.: Z01 CM 06921-02 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Adriamycin Therapy for Advanced Mycosis Fungoides

Previous Project Number: Same

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: Larry Kvols, M.D., Robert A. Slawson, M.D.

Cooperating Units: Section of Radiotherapy, University of Maryland Hospital

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides.

Adriamycin was given to patients with a biopsy proven diagnosis of mycosis fungoides who were admitted to the BCRC. All patients had received previous therapy and were now refractory. Disease status was advanced, extensive skin disease, lymphadenopathy was present in all cases. Adriamycin was administered in a dose of 60 mg/m^2 IV every 3 weeks and doses modified if any liver dysfunction or significant myelosuppression occurred. A minumum of 3 courses were given and if complete response ensued, a further 2 courses beyond complete response given. Maintenance therapy consists of methotrexate 15 mg/m^2 IM twice weekly and cytoxan 750 mg/m^2 IV every 3 weeks.

Eight patients have been entered into this study and 7 of these are evaluable at this point. There has been one complete response, 4 partial responses and 2 failures. The median duration response has been 11 weeks (range 10 - 55+ weeks) and the duration of response for the patient who entered into complete remission has been 32 weeks. Toxicity has been mild and only a moderate degree of myelosuppression encountered. It therefore appears that adriamycin is effective in the treatment of advanced mycosis fungoides.

Mycosis fungoides is an unusual form of lymphoma which often has a slow course early in its stages of development. However, once the disease is advanced, there is often rapid progression and no form of chemotherapy used to this time has improved survival. Adriamycin appears to be an effective agent and if intensive therapy with this drug can produce a number of responses and prolong duration of response and survival time, then further studies with this agent alone and in combination with others may improve the outlook for patients with this distressing disease.

Keyword Descriptors: Mycosis fungoides, chemotherapy

Honors and Awards: None

Publications:

1. Levi, J., Wiernik,P.: Management of Mycosis Fungoides - Current Status and Future Prospects. Medicine 54: 73, 1975.

Project No. Z01 CM 06922-03 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Md.

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title:	Epidemiology Investigations of 1) Prior Social Interaction Among Patients with Leukemia and Lymphoma and 2) the Possible Association between Environmental Solvents and Cancer Induction.
Previous Serial Number:	NCI 6922
Principal Investigator:	Stephen C. Schimpff, M.D.
Other Investigators:	John Wolff, M.D., Dorah Brager, B.A., Peter H. Wiernik, M.D.
Cooperating Units:	George Comstock, M.D., Johns Hopkins, School of Public Health. Frances Shaver, RRL, West Virginia Cancer Control Bureau, Charleston, West Virginia. Malcolm Pike, Ph.D., University of California at Los Angeles. Barry Friedlander, M.D., Dept. of Cancer Control, Maryland State Department, Department of Health.

Man Years

Total	2
Professional	1
Other	1

Project Description

A) Prior Social Interaction Among Patients with Leukemia and Lymphoma - Both leukemias and lymphomas have variously been suggested as diseases possibly caused by an infectious agent. Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of interpersonal contact in the epidemiology of both leukemia and lymphoma. Each case report of leukemia and lymphoma for a 10 year period from three areas of West Virginia was obtained and the patient or a surviving friend or relative was interviewed. In each area, over 60% of all cases could be interlinked on the basis of prior close personal interactions. Similar findings were obtained from two areas in Maryland and one in Pennsylvania. Twenty eight patients with Hodgkin's disease from various areas in the USA were selected at random and interviewed; ten had a history of close social linkages to other individuals with leukemia or lymphoma. Finally, a case-control approach was utilized in one areaiin which all patients for a ten year period were matched to normal controls. After all patients and controls were interviewed, it was found that the patients had statistically significantly more close personal associations between themselves than did the matched controls. The study continues.

B) Evaluation of Environmental Factors in Cancer Epidemiology - The BCRC has been involved in an investigation of a possible cluster of lymphoproliferative diseases in Providence Valley, Maryland. Other agencies have investigated the extent of environmental pollution in the area while the BCRC has investigated the clinical material. Providence Valley is a small geographic area with an organic solvent reclamation plan in its center. Malodorous vaporous wastes appear to accumulate in the valley because of the surrounding hills. A pathologist in nearby Elkton, Md. suggested that there was an apparent increase in cancer deaths among the 400 residents of the Valley. The BCRC has examined a substantial portion of the resident population and conducted studies on their blood and urine in conjunction with environmental studies conducted by the State Health Department. The investigation is still in progress and will require further evaluation before specific findings can be reported.

Keyword Descriptors: Epidemiology, leukemia and lymphoma.

Publications:

Schimpff, S.C., Schimpff, C.R., Brager, D.M., Wiernik, P.H.: Leukemia and lymphoma patients interlinked by prior social contact. Lancet 1: 124-129, 1975.

Project No.: Z01 CM 06928-01 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: High Dose Methotrexate (HDMTX) for the Therapy of Advanced, Refractory Hodgkin's Disease, non-Hodgkin's Lymphoma and Solid Tumors

Previous Project Numbers: None

Principal Investigators: Charles A. schiffer, M.D., John A. Levi, M.D.
Peter H. Wiernik , M.D.

Other Investigators: Stephen D. Reich, M.D., Nicholas Bachur, M.D.

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

1. To determine the efficacy of high dose methotrexate in patients with advanced, refractory lymphoma and solid tumors.
2. To determine the feasability of administrating high dose methotrexate to patients who have undergone extensive prior therapy.

Only patients with normal creatinine clearances were eligible for the study. An initial starting dose of 100 mg/kg administered intravenously over a six hour period was employed. This was followed by citrovorum factor, 9 mg IM q6h x 8 doses to be followed by 9 mg po q6h x 4 doses. The CF was begun two hours after the methotrexate infusion was terminated. Because of severe toxicity noted in two of the patients treated initially, the citrovorum dose was increased so as to equal 10% of the total dose of methotrexate administered.

A total of 14 patients have been treated to date. Significant antitumor responses have been seen in two patients with acute lymphoblastic leukemia. Responses were of short duration, however, and neither patient achieved a complete response. One patient with stable metastatic osteogenic sarcoma has remained stable for the past six months while receiving high dose methotrexate. Severe muscosal and hematopoietic toxicity was seen in two patients earlier in the study. In at least one of these patients, toxicity may have been related to impaired secretion of the drug due to renal obstruction. Toxicity has been tolerable in the last 10 patients treated. Pharmacokinetic studies have revealed that peak levels of approximately 10^{-3} to 10^{-4} M are achieved for very short periods of time following the infusion. Most of the drug is excreted in the urine in the first 24 hours. This study demonstrates that with careful selection of patients, methotrexate can be administered with safety in massive doses. Further studies are necessary to determine means of predicting and preventing the severe toxicity which occasionally occurs.

Further pharmacokinetic analyses will be performed in an effort to determine the appropriate timing and dose of citrovorum factor.

Keyword Descriptors: Citrovorum factor "rescue", High dose methotrexate

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06929-01 BCRC
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Pilot Study of Combination Therapy with 5-azacytidine, and methyl GAG in Previous Treated Adults with Acute Nonlymphocytic Leukemia

Previous Project No.: None

Principal Investigators: John A. Levi, M.D., Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

To determine whether the combination of 5-azacytidine and methyl GAG could improve on the results achieved with 5-azacytidine alone and whether administration of methyl GAG as a continuous IV infusion will reduce the incidence of serious toxicity previously reported with this drug.

Eight adults with acute nonlymphocytic leukemia who have received extensive prior therapy were included in this study. Only one patient achieved a partial remission on this regimen and one other achieved definite improvement in bone marrow status without approaching a remission. Survival from the onset of therapy was brief for the remaining six patients (2 to 8 weeks). All patients treated with this regimen suffered definite and often severe nonhematologic toxicity. The addition of methyl GAG resulted in an increase in both the number and degree of severity of toxic effects previously described with 5-azacytidine alone. Mucosal toxicity, in particular, was a serious problem and could be directly related to subsequent infection and death of four patients. Because of the poor responses and severe toxicity encountered with this regimen, it is felt that this combination is not suitable for further study. It also seems that no advantage could be obtained from the continuous IV infusion of methyl GAG and this drug in general remains too toxic to use in induction of acute leukemia.

Study closed to patient entry and other combination studies with 5-azacytidine have begun.

Project No.: Z01 CM 06929-01 BCRC

Keyword Descriptors: Acute leukemia, chemotherapy

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06024-09 BCRC
1. Baltimore Cancer Research Center
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of a Neurosurgical Service with Primary Interest in the Treatment of Brain Tumors at the BCRC, NCI

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators : Philip H. Gutin, M.D.
Marshall A. Levine, M.D.
Edward A. Neuwelt, M.D.
Ronald Cohen, M.D.

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

This ongoing project is designed to develop an effective research Neurosurgical Service to evaluate various modalities of treatment for intracranial neoplasms. Pilot studies are performed to evaluate the efficacy of drugs in relation to Phase I-II and neuropharmacologic studies. The profile of this service has changed and been enhanced during the past year following the move of the BCRC to the University of Maryland Hospital, by virtue of its association with an ongoing general neurosurgical service and the availability of high-quality neuro-diagnostic services. Interdisciplinary studies of blood-brain barrier, pharmacology cellular membrane permeabilities and clinical brain tumor chemotherapy interrelate the activities of the clinic and those of the laboratory.

Patients with intracerebral neoplasms are referred to the BCRC Section of Neurological Surgery for combined modalities therapy. The patient population is referred from the Greater Baltimore Medical Community, as well as the UMH Services. Clinical and pharmacologic evaluation of chemotherapeutic agents are one of the principal goals of the Service, and is carried on in combination with operative intervention. Previous studies indicated the efficacy of intravenous MeCCNU and epipodophyllotoxin. A Phase II study utilizing both these drugs in relation to radiotherapy is being carried out and nearing completion. Intravenous CCNU has been carried through Phase I and is being considered for further evaluation in Phase II. The Neurosurgical Service also participates in the Brain Tumor Study Group.

Close collaboration with the Medical Oncology Service of the BCRC is maintained in order to develop new approaches to the treatment of meningeal neoplasia. Phase I studies of intrathecal thiotepa have been completed and suggested efficacy shown. Phase II studies are currently underway to definitively characterize the clinical value of intrathecal thiotepa. Corticosteroids have shown clinical efficacy in the treatment of cerebral edema related to brain tumor, as well as oncolytic effects in other tumors. Therefore, high dose corticosteroid treatment of intracranial neoplasm is being evaluated as a possible mode of therapy for the treatment of this disease.

As the multimodality therapeutic effect of extra CNS neoplasms becomes more of a reality, metastatic disease to the brain is becoming a more important obstruction to the control of this disease. Longer survivals allow metastasis to the central nervous system so that effective drug therapy in this area of secondary CNS involvement is becoming imperative. This is well demonstrated in CNS leukemia and lymphoma.

The Neurosurgical Service of the BCRC will continue to admit, diagnose and treat operatively, chemotherapeutically and radiotherapeutically patients with intracranial malignancy. Continued Phase I-II studies of new chemotherapeutic agents with appropriate pharmacokinetic characteristics for successful brain tumor chemotherapy will be undertaken. In addition, pharmacologic studies in man will continue to be pursued.

Keyword Descriptors: brain tumor, malignant glioma, CNS neoplasm, nitrosoureas, blood-brain barrier, pharmacokinetics.

Honors and Awards:

Publications:

Merker, P.C., Walker, M.D., Richardson, E.P., Jr. and Shepard, R.F.: Neuropharmacological Effects of Anticancer Drugs Perfused Through the Cerebrospinal Fluid System of the Rhesus Monkey: Cytosine Arabinoside. Excerpta Medica Int. Congress Series No. 311, 15: Jurich, June, 1973.

Sklansky, B.D., Kaplan, R.S., Reynolds, A.F., Rosenblum, M. and Walker, M.D.: 4'Demethyl-Epipodophyllotoxin-B-D-Thenylidene-Glucoside (PTG) in the Treatment of Primary Intracranial Neoplasms. CANCER 33: 460-467, 1974.

Walker, M.D.: Glioblastoma Multiforme. Neurosurgical Forum. J. Neurosurg. 41: 405-406, 1974.

Weiss, H.D., Gutin, P.H., and Walker, M.D.: Unresolved Problem of Treatment of Glioma. JAMA 229: 1284-1285, 1974.

Weiss, H.D., Walker, M.D. and Wiernik, P.H.: The Neurotoxicity of Commonly Used Antineoplastic Agents. New Engl. J. Med. 291: 75-81, 127-133, 1974.

NCI-06024

Walker, M.D.: Adjuvant Chemotherapy. Seminar in Oncology Vol. II, No. 1, 69-72, March 1975.

Project No. Z01 CM 06204-04 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Toxicity of Intracerebral Chemotherapy in Monkeys

Previous Serial Number: Same

Principal Investigators: Michael D. Walker, M.D.
Philip H. Gutin, M.D.
Archie Bleyer, M.D.

Other Investigators: Dan Dalgard, D.V.M.

Cooperating Units: Hazelton Laboratories, Falls Church, Virginia

Man Years:

Total:	.1
Professional:	.1
Other:	.0

Project Description:

With the increase in meningeal leukemia and the interest in neoplasms within the central nervous system, the use of intrathecal chemotherapy is expected to continue to increase. In this ongoing project, a relatively standardized technique for evaluating intrathecal chemotherapy in monkeys is being employed and provides a resource for rapid evaluation of new concepts and ideas as they develop. The project is being used to test a specific agent of choice, rather than to generally screen chemotherapeutic drugs; therefore, this research operates on a demand basis.

Intracisternal installation of carefully chosen drugs are first performed to determine acute immediate toxicity. From this information, dose levels for chronic studies simulating the dose schedules used in man are developed. Previously, the use of intrathecal Ara-C was defined in this project, and more recently, the data necessary for the use of intrathecal thiotepa has been developed. The Phase I Study in man authenticated the findings in monkey. The project has been in a holding pattern during the course of this year, however, we will undertake the evaluation of an additional chemotherapeutic agent in the forthcoming year.

Keyword Descriptors:

Intrathecal drug toxicity; Ara-C; ThioTEPA, Meningeal neoplasm

Project No. Z01 CM 06282-09 BCRC

1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 212 1

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Brain Tumor Study Group

Previous Serial Number: Same

Principal Investigators: Michael D. Walker, M.D.
Thomas A. Strike, Ph.D.

Other Investigators: Patricia T. Lutz, B.S.
Members of the Brain Tumor Study Group

Cooperating Units: Members of the Brain Tumor Study Group

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description:

The Brain Tumor Study Group (BTSG) was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of malignant gliomas utilizing Phase II type studies. The goals of the BTSG are 1) to increase the number of patients that respond to treatment, 2) prolong the period of remission and 3) increase the life expectancy of these patients. The program has been broadened to include evaluation of radiation therapy as a modality alone and in combination with chemotherapy. Additionally, the BTSG is also conducting Phase II studies in order to make a more meaningful selection of chemotherapeutic agents for future Phase III studies.

Members of the Brain Tumor Study Group have established a cooperative, ongoing and continuous effort providing patients for multitherapeutic modality studies which utilize a prospective, controlled, randomized study design. Patients diagnosed for the first time as having malignant gliomas are randomized to the Phase III study shortly after definitive surgery. Patients with recurrent gliomas or other types of brain tumors are randomized to the Phase II study. Final data analysis of the previous Phase III protocol evaluating BCNU and irradiation alone and in combination has been completed and the study report is in preparation. The current Phase III protocol is designed to evaluate methyl CCNU alone and in combination with radiation; radiation alone; and BCNU in combination with radiation. This study is approaching the point where a preliminary analysis of the data can be made. In addition, the program has broadened to include the investigation of four new drugs in a cross-over Phase II protocol design. Adriamycin, Dibromodulcitol, Procarbazine, and Streptozotocin are being evaluated in patients with recurrent malignant glioma. Patients deemed failures to therapy with one of the aforenamed drugs are rerandomized to either a high tapering dose of corticosteroids, or a moderate prolonged course of corticosteroid. Administrative support is provided by the Central Office, and institutional support by the contract mechanisms. Semi-annual meetings of the Brain Tumor Study Group and staff visits from the Central Office provide appropriate communication and exchange of information.

The BCNU/irradiation protocol had 303 patients randomized into its four arms, of which over three quarters are considered within the valid study group. Final evaluation of the data is underway. However, an intermediate evaluation indicates that surgical therapy alone provides a median survival of 14 weeks, BCNU a median survival of 21 weeks, radiotherapy a median survival of 31 weeks, and BCNU plus irradiation a median survival of 41 weeks.

The ongoing BCNU/methyl CCNU/irradiation Phase III protocol is approximately three quarter complete in its patient accrual and a preliminary analysis of the existing data is underway.

The Phase II studies have accrued over 30 patients on each arm. However, there are too few patients in the study for a data analysis.

The BTSG held a multidisciplinary meeting in the Fall in which scientific papers on neuropathology, brain scan techniques, and irradiation physics were presented. This meeting provided a major focus for interdisciplinary activities. The Spring meeting consisted of a business session and a scientific seminar titled "Immunotherapy Approaches in the Treatment of Brain Tumors".

These studies are the only prospecitve, controlled, randomized studies performed in the United States in the Treatment of brain tumor. This project provides specific detailed and timely information concerning the efficacy of various therapeutic modalities for the malignancy of the central nervous system. This project is also in keeping with both the long range goal and the short term objectives of the Division of Cancer Treatment Program.

A final analysis of the BCNU-irradiation study is being undertaken and a report of the study results are being prepared. The BCNU; methyl CCNU/irradiation protocol will continue to accrue patients and is expected to be closed to accrual early FY '76. From the four Phase II drugs currently under study, it is expected that one or two of these agents will be chosen for the next controlled, prospective, randomized Phase III study of the group.

The BTSG also is expanding its membership in order to perform these studies more rapidly. Several new members are expected to be added to the BTSG before the end of FY '75.

Keyword Descriptors: Brain Tumor, malignant glioma, chemotherapy, radiation

therapy, Phase II trials, Phase II trials, nitrosoureas.

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06904-03 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on the Anti-Tumor Activity of Methyl-CCNU and Related Compounds at the Subcellular Level.

Previous Serial Number: Same

Principal Investigator: John Hilton, D. Phil.

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.
Deborah Zito, Student

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.6
Others:	0.3

Project Description:

The anti-tumor agent CCNU has the potential for alkylation of DNA, although the specifics have never been investigated. This project has extended into this area to determine the fate of cellular DNA after treatment of L1210 and rat glioma cells with CCNU both *in vitro* and *in vivo*. Our preliminary data indicates that exposure of cells to CCNU leads to the production of single strand breaks in DNA which can be demonstrated by analysis of the DNA or alkaline sucrose gradients or by hydroxylapatite chromatography. The latter, more sensitive method of analysis has detected the production of single strad breaks in DNA at CCNU concentrations well within the physiologically realistic range. In a study of this nature, the cell's ability to repair single strand breaks in DNA and the ability of the alkylating agent to produce these breaks are of equal importance. So far we have been to demonstrate repair of CCNU induced damage while knowing that, for example, gamma-irradiation damage is quickly repaired. Our data indicate that CCNU may act both to produce damage in DNA and to inhibit the cells repair process. Future work will examine this hypothesis both at the cellular level and in whole animal studies (Project Z01 CM 06903-01 BCRC).

Keyword Descriptors: nitrosourea, alkylation, DNA, alkaline sucrose gradients, hydroxylapatite chromatography, DNA repair, L1210, rat glioma.

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06906-02 BCRC

1. BCRC
2. Section of Neurological Surgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intravenous CCNU Phase I Study

Previous Serial Number: Same

Principal Investigator: Marshall A. Levine, M.D.

Other Investigators: Michael D. Walker, M.D.
Peter H. Wiernik, M.D.

Cooperating Units: Medical Service, BCRC, NCI, DCT

Man Years:

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Nitrosoureas have been utilized for a variety of neoplastic diseases with some therapeutic efficacy. However, the irregular and unpredictable toxicity and the often low level of efficacy have led to modifications of the molecular structures and delivery techniques of the nitrosoureas. An intravenous preparation for MeCCNU required a cumbersome administration technique. Emulfor, a non-ionic polyethoxylated castor, has been used for the administration of PTG and recently has been adapted for the use of IV-CCNU. This study evaluated the toxicity of IV-CCNU using the emulfor vehicle for administration.

Using the vehicle, 5% ethanol and 5% emulfor in D5W, patients were administered IV-CCNU starting at 25 mg/M^2 with doses increasing by 20 to 30 mg/M^2 increments as suitable toxicity levels were found. Patients entered had acceptable characteristics for Phase I-II trials.

Essentially no toxicity was noted at 25, 50 and 75 mg/M^2. At 100 mg/M^2, 30% of the patients developed moderate or severe toxicity. At 130 and 150 mg/M^2, 55% of the patients developed moderate or severe toxicity. At 170 mg/M^2, nearly all patients developed moderate or severe toxicity. Early and late toxicity occurs using this drug. The early toxicity consists of nausea, vomiting, diarrhea, and chills and fever to 102°. This early toxicity occurs after 6-12 hours and last 3-4 hours. Additionally, dyspnea and/or hypoxia have been noted following administration in patients receiving 100 mg/M^2 or higher. Hematologic toxicity appears to be dose limiting, occurs after 3-4 weeks lasting 1-2 weeks, effects platelets more severely than the hematocrit and white count, and is cumulative although unpredictable. Shrinkage of masses in patients with lung and colon neoplasms were noted and Phase II trials have been undertaken.

A simple means of delivering an aqueous insoluble compound intravenously has been administered. The drug does seem to have some efficacy in colon cancer. Phase II studies are presently underway in colon cancer and other neoplasms.

Keyword Descriptors: Nitrosoureas, toxicity, Emulfor, IV-CCNU, Phase I-II trials, chills and fevers, dyspnea and hypoxia, hematologic toxicity, efficacy, colon, dose limiting.

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06907-02 BCRC
1. BCRC
2. Section of Neurological Surgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Colony Stimulating Factor (CSF) and Colony Forming Capacity (CFC) from Patients with Malignant Neoplasms, and the Effects of Chemotherapy upon CSF and CFC.

Previous Serial Number: Same

Principal Investigator: Marshall A. Levine, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Michael D. Walker, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Since the 1960's, numerous reports of the successful in vitro growth of human peripheral blood and bone marrow have been published. In small groups of leukemia patients, CSF and CFC have been shown to rise during remission and fall during relapse. In addition, leukemic cells have been shown to mature in vitro on occasion. The large leukemic population of the Baltimore Cancer Research Center would allow more adequate assessment of these findings. Additionally, the mechanism of delayed cumulative hematologic suppression with nitrosourea therapy has not been explained. The in vitro culture technique would allow us to determine if decreased CSF (stimulatory) or decreased CFC (target) is the basic deficit caused by nitrosourea chemotherapy. The large malignant central nervous system neoplasm patient population of the BCRC would allow a unique opportunity to study this peculiarity of the nitrosoureas. Primary central nervous system neoplasm patients rarely have metastases to bone marrow producing areas. The following assessment will be made:

1. Measurement of CSF and CFC in various stages of hematologic malignancy.
2. Measurement of CSF and CFC in various stages of solid tumors infiltrating bone marrow.
3. Measurement of CSF and CFC in patients in various stages of chemotherapy, particularly with nitrosourea compounds.

4. Morphologic studies of in vitro grown leukemic cells.

The growth technique involves incubation for a short period of time a source of CSF such as urine, peripheral white blood cells, plasma or serum in McCoy's media, fetal calf serum, and dilute agar or methyl cellulose. Bone marrow from human beings or mice are evenly dispersed in the same semisolid culture medicum and incubated over the prepared feeder layers. The cultures are incubated in 35 mm petri dishes for 10-21 days in a humidified CO_2 incubator at 37°C, removed and examined under a dissecting microscope. Culture dishes are examined for colony counts and cell morphology.

The project is in its early stages. More consistency in the growth of cells is required at this time. In vitro studies using this technique will help in the understanding of the mechanisms of hematologic malignancies. Additionally, it is hoped to develop better understanding of the unusual delayed, cumulative toxicity of nitrosoureas. Further refinements in the culture techniques are anticipated. When consistent growth is obtained, detailed studies of hematopoietic fucntion will be undertaken. We also hope to develop some information regarding tumor burden levels in the bone marrow.

Keyword Descriptors: colony stimulating factor, CSF, colony forming capacity, CFC, leukemia, delayed cumulative hematologic toxicity, nitrosourea chemotherapy, bone marrow, culture.

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06908-02 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intravenous Methyl-CCNU (NSC-95441) and PTG (NSC 122819) in the Treatment of Central Nervous System Neoplasms (Phase II).

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Philip H. Gutin, M.D.
Marshall A. Levine, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.3
Professional:	0.3
Other:	0

Project Description:

Methyl-CCNU and epipodophyllotoxin (PTG) have been used singly with apparent efficacy in central nervous system tumors. In this study their use in combination is being evaluated. Patients with biopsy proven or clear cut clinical evidence of intracerebral malignancy are given intravenous methyl-CCNU 130 mg/M^2 every 6-8 weeks and PTG 100 mg/M^2 weekly. Many patients also receive concomitant cranial irradiation. Bone marrow toxicity is monitored closely with complete blood counts and platelet counts done twice weekly. SMA-12 profiles are done on a weekly basis as well. Twenty patients have received this regimen to date and of these 15 have received one full course of treatment and are evaluable. Three patients deteriorated within two courses of therapy; eleven continued in a state of well being for 1+, 10+ (median 5 months) months and one patient stabilized from progressing disease for 5 months. Delayed cumulative bone marrow toxicity was profound with 8 of 17 patients having thrombocytopenia (less than 50,000) and leukopenia (less than 2,000). Thus far, then, methyl-CCNU and PTG do not appear to have enhanced toxicity when used in combination. We plan to complete this study with the addition of several more patients within the next few months.

The use of combinations of agents have been important in the development of treatments against non-essential nervous system neoplasia. This study is one of the first attempts to use combinations of agents against central nervous system tumors.

Project No.: Z01 CM 06908-02 BCRC

Kayword Descriptors: glioma, PTG, Methyl-CCNU, combination chemotherapy, brain tumor chemotherapy.

Honors and Awards: None

Publications: None

Project No.: Z01 CM 06909-02 BCRC
1. BCRC
2. Section of Neurological Surgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Phase II Evaluation of Intrathecal N,N',N"-Triethylenethiophosphoramide (Thiotepa-NSC-6396) in Meningeal Neoplasms.

Phase I Evaluation of Thiotepa was closed June 30, 1974. This is the continuing clinical evaluation of this agent.

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Philip H. Gutin, M.D.
John A. Levi, M.D.
Peter H. Wiernik, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	0.3
Professional:	0.3
Other:	0

Project Description:

Following a complete Phase I evaluation of intrathecal thiotepa it was determined that dosages up to 10 mg/M^2 were tolerated with no marrow or central nervous system toxicity. Continuing use of this agent against meningeal neoplasms of all types is now underway in an effort to clearly evaluate the clinical efficacy of this agent. Adult patients receive 10 mg intrathecally every second day while children below 10 years receive 6 mg. Close hematological and neurological evaluation continues to further evaluate any unforeseen toxicity. Thus far 5 patients have been treated under the Phase II regimen; and although it is early to evaluate responses, it seems that two of these patients have had some anti-tumor effect. Of the patients treated thus far, one has had ALL, two have had meningeal carcinomatosis and two have had AML.

It is obviously important to develop agents for use against meningeal neoplastic disease since the current armamentarium of agents is extremely small and of limited efficacy.

Project No.: Z01 CM 06909-02 BCRC

Keyword Descriptors: meningeal leukemia, meningeal carcinomatosis, meningeal lymphoma, thiotepa, intrathecal chemotherapy.

Honors and Awards: None

Publications: The Phase I study of this agent is now in preparation for publication.

Project No. Z01 CM 06910-03 BCRC
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland 21201

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: *In Vitro* Metabolism of CCNU and Methyl CCNU

Previous Serial Number: SAME

Principal Investigator: John Hilton, D.Phil.

Other Investigators: Michael D. Walker, M.D.
Donald Bowie, B.S.
Deborah Zito

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

Rat liver microsomal mixed function oxidase catalyses the hydroxylation of the cyclohexyl moiety of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) to give at least five metabolites. When exposed to alkaline pH at 100°, CCNU and its metabolites quantitatively release their cyclohexyl moiety as cyclohexylamine and aminocyclohexanol, respectively. The N-(2,4-dinitrophenyl) derivatives of cyclohexylamine and aminocyclohexanols were separated by high pressure liquid chromatography. The *in vitro* metabolites have been identified as *trans*-2-hydroxy CCNU, *cis*-3-hydroxy CCNU, *trans*-3-hydroxy CCNU, *cis*-4-hydroxy CCNU and *trans*-4-hydroxy CCNU. Ring hydroxylation axial to the 1-(2-chloroethyl)-1-nitrosourea group (*cis*-2-, *trans*-3-, *cis*-4-) is favored over equatorial attack (*trans*-2-, *cis*-3-, *trans*-4).

This project is progressing toward the quantitative preparation of stable derivatives of the nitrosoureas and their hydroxylated metabolites in order to facilitate future pharmacological studies in experimental animals and man.

Keyword Descriptors: nitrosourea; mixed function oxidase; enzymic hydroxylation; high pressure liquid chromatography.

Publications:

Hilton, J. and Walker, M.D.: Hydroxylation of 1-(2-chloroethyl)-3-Cyclohexyl 1-Nitrosourea. Biochem. Pharmacology (in press)

Project NO.: Z01 CM 06911-03 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: In vivo Metabolism of 3-cyclohexyl substituted 1-chloroethyl-1-nitrosoureas

Previous Serial Number: Same

Principal Investigator: John Hilton, D. Phil.

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.
Deborah Zito, Student

Cooperating Units: None

Man Years:

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

CCNU given i.v. to male Fischer rats is rapidly hydroxylated on the cyclohexyl ring to give at least five metabolites. When exposed to alkaline pH at 100° CCNU and its metabolites quantitatively release their cyclohexyl moiety as cyclohexylamine and aminocyclohexanol, respectively. The N-(2,4-dinitrophenyl) derivatives of cyclohexylamine and aminocyclohexanols were separated by high pressure liquid chromatography. The in vivo metabolites have been identified as trans-2-hydroxy CCNU, cis-3-hydroxy CCNU, trans-3-hydroxy CCNU, cis-4-hydroxy CCNU and trans-4-hydroxy CCNU. Ring hydroxylation axial to the 1-(2-chloroethyl)-1-nitrosourea group (cis-2-,trans-3-, cis-4-) is favored over equatorial attack (trans-2-, cis-3-, trans-4-). Pretreatment of rats with phenobarbital leads to an increased rate of hydroxylation and a change in the relative amount of the hydroxylated products.

Plasma samples from several patients receiving CCNU chemotherapy have been analyzed for parent drug and hydroxylated metabolites up to 8 hours following therapy. The major portion of theplasma nitrosourea is always present as hydroxylated metabolites which has been identified as cis-4-hydroxy CCNU and trans-4-hydroxy CCNU.

A study of tissue distribution of CCNU and its metabolites is planned with a view to determining the pharmacologic and therapeutic consequence of CCNU metabolism.

Project No.: Z01 CM 06911-03 BCRC)

Keyword Descriptors: Nitrosourea, enzymic hydroxylation, high pressure liquid chromatography, clinical pharmacology.

Honors and Awards: None

Publications:

Hydroxylation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. in press, Biochemical Pharmacology.

Project No.: Z01 CM 06930-01 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Alkylation of DNA in the Rat Glioma Model by Intravenous CCNU

Previous Serial Number: None

Principal Investigator: Philip H. Gutin, M.D.

Other Investigators: John Hilton, Ph.D.
Michael D. Walker, M.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

CCNU is an effective agent in the treatment of human malignant glioma but its mode of action is obscure. Chemical studies have suggested alkylation as a possible mechanism. Currently we are perfecting the rat intracerebral glioma model in our laboratory. This will serve as the substrate for DNA analysis following CCNU therapy. Preliminary studies will be done to determine CCNU toxicity in non-tumor bearing rats and efficacy in rats bearing tumors. Rats with intracerebral glioma will then receive intravenous CCNU in sublethal yet efficacous doses. At various intervals following therapy, tumors will be quickly removed from the brain and gently squashed to separate cells. Cell suspensions will then be layered onto alkaline sucrose gradients and then placed in an ultracentrifuge. The gradients will be drained into fractions and the DNA in these fractions will be analyzed by a fluorometric assay. This method of looking at DNA integrity has been well worked out in other systems. The migration of DNA down the sucrose gradient depends on its integrity. Heavier (native) DNA which has not been alkylated will move toward the bottom of the gradient while alkylated DNA will divide into single stranded fragments and appear in a random fashion in the middle of the gradient.

This work is important as it is one of the few studies to determine the effects of nitrosoureas on tumor tissue. It may lead to the ability to evaluate the response of a brain tumor to an administered agent.

The model should be perfected within the next two or three months and then early next year the alkylation data should be accrued.

Keyword Descriptors: CCNU, alkylation, DNA analysis, glioma, alkaline sucrose gradients, intracerebral glioma model, nitrosoureas, brain tumor, agent

Honors and Awards: None

Publications: None

Project No: Z01 CM 06931-01 BCRC
1. BCRC
2. Section of Neurosurgery
3. Baltimore, Maryland 21201

PHS - NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Intravenous CCNU Phase II Study in Colorectal Adenocarcinoma

Previous Serial Number: None

Principal Investigator: Marshall A. Levine, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Joseph Aisner, M.D.

Cooperating Units: Section of Medical Oncology, BCRC, NCI, DCT

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Colorectal adenocarcinoma has proven to be a neoplastic disease unresponsive to chemotherapy. Advance colorectal adenocarcinoma has a 2-6 month prognosis with less than 20% of the patients responding to any modality of systemic therapy. IV-CCNU showed some efficacy in several early patients with colorectal adenocarcinoma. Consequently, a formal Phase II trial was undertaken.

130 mg/M^2 of CCNU was administered using a vehicle of 5% emulfor and 5% ethanol in D5W. Patients entered were generally untreated although several had received radiation therapy or 5-Fluorouracil chemotherapy. Patients were treated until definite tumor progression occurred.

The principal toxicities of IV-CCNU therapy were again noted in this Phase II trial. Proven toxicity consisted of nausea and vomiting and diarrhea, chills and fever to 102°, and dyspnea with hypoxia to 55-60 mm. These were noted in many patients. The late toxicity consisted of thrombocytopenia occurring at 3-4 weeks and lasting 1-2 weeks, and leukopenia and anemia occurring at 4-5 weeks and lasting 1-2 weeks. Initially, dose limiting toxicity appeared to be 130 mg/M^2, but this was subsequently reduced to 100 mg/M^2 because of more severe hematologic toxicity than occurred in the

Phase I study of IV-CCNU.

To date, 13 patients are evaluable and 3 are presently too early following therapy to give a definite determination of therapeutic efficacy. Of 13 evaluable patients at present, 2 patients have shown definite partial remissions and a third a probable partial remission. Three additional patients have shown 25-50% improvement or stabilizations of previously advancing disease. Seven patients have shown progression of disease following IV-CCNU therapy. It is our impression that this drug does have efficacy in colorectal adenocarcinoma but that the early toxicity of chills, fever, dyspnea and hypoxia is more profound than was appreciated initially.

Phase II and Phase III studies using IV-CCNU in combination with other chemotherapeutic and immunotherapeutic agents in anticipated.

KeyWord Descriptors: Phase II trial, intravenous CCNU, IV-CCNU, colorectal adenocarcinoma, emulfor and ethanol in D5W, toxicity, chills and fever, hematologic toxicity, dyspnea and hypoxia, efficacy.
Honors and Awards: None

Publications: None

Project No. Z01 CM 06043-05 BCRC
1. BCRC
2. Clinical Research Pharmacy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Automated Monitoring of Drug Therapy

Previous Serial Number: 6043

Principal Investigators: Clarence L. Fortner, James K. Hooper

Other Investigators: Michael D. Walker, Richard Hsieh, William R. Grove Peter H. Wiernik

Cooperating Units: Division of Health Services Research, Baltimore USPHS Hospital; Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 2½
Professional: ½
Other: 2

Project Description:

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing four copies: the medicine card copy, the pharmacy copy (for entry to a patient drug profile), and copies for medical records and automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration of omission of each drug. A monthly drug utilization report is also obtained as a by-product.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each *patient. Space on the drug form has also been allotted for the patients' height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.*

Keyword Descriptors: Computerized Drug Data, Investigational Drug Data, Patient Medication Records.

Project No. Z01 CM 06236-05 BCRC
1. BCRC
2. Clinical Research Pharmacy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

Previous Serial Number: 6236

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and John F. Bender

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1/8
Professional: 1/8
Other: 0

Project Description:

The objective is to evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a complete drug and allergy history.

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart and in the pharmacy's drug profile for each patient.

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of admission-92%, and patients reporting a history of allergy to drugs, food, animals, clothing, tape and iodine-31%. The interview includes determining all of the over the counter (OTC) drugs that the patient has received without professional supervision. This information is extremely valuable at the time the patient is discharged from the hospital. The pharmacist is able to utilize this information by advising the patient of any drug-drug interactions, etc. between the OTC drug and drugs being prescribed by the physician *upon discharge. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the hospitalized period. The pharmacists are responsible for placing all known allergic reaction information on the front of the patients drug Kardex, and on the problem sheet in the patient's chart. At the time of completion of the interview, the original is placed in the history section of the patients hospital chart and a duplicate is attached to the patients drug profile in the pharmacy. If the pharmacists feel that some of the information should be brought to the physicians attention immediately, he also communicates verbally to the physicians. These histories are also utilized at the outpatient window as the patient receives outpatient medication. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service. The drug and allergy interview by the pharmacist has become an integral part of the clinical pharmacy program. The present study will be continued.*

Keyword Descriptors: Drug History, Allergy History

Project No. Z01 CM 06237-06 BCRC
1. BCRC
2. Clinical Research Pharmacy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of a Clinical Research Pharmacy Service

Previous Serial Number: 6237

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and Robert R. Foreman

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1-1/3
Professional: 1-1/3
Other: 0

Project Description:

The pharmacist has traditionally been a product oriented person with little input to the actual care of the patient. The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care. Patient education has been added to this study. The objective of this education will be to increase compliance and reduce problems for outpatients receiving drugs.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patients' condition. Therefore he can intelligently discuss therapy problems with the patient's physician. With this information, he can also provide the staff with drug information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile. The drug profile includes the patient name, primary and secondary problems, physician name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems and possible adverse drug reactions. The drugs are then dispensed thru a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system involves evaluating the necessity of continued medication orders.

An extensive library, including an drug abstracting service, aids the pharmacist in performing an additional service to the BCRC, one of drug information. Physicians questions are researched and answered promptly, to aid in their ability to treat the patient.

The pharmacist has showed that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and contributes directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug-induced laboratory test abnormalities, detecting possible drug-induced diseases, providing drug information, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better patient care and has enhanced the quality of the research data.

Present studies will be continued and additional methods to improve patient care, will be further researched.

Honors and Awards: American Pharmaceutical Association Federal Pharmacy Literary Award, 1974 (Hodgkin's Disease: Journal of the American Pharmaceutical Association: Vol. NS13, No. 3:147-154 (March) 1973).

Publications: DeChristoforo, R. and Fortner, C.L.: A Review of Potential Drug Interactions Between OTC and prescription Drugs; Que Newsletter, Upjohn, Issue 4, 1974.
Schimpff, S.C., Fortner, C.L., Greene, W.H. and Wiernik, P.H.: Cytosine Arabinoside for localized Herpes Zoster in Patients with Cancer: Failure in a Controlled Study; Journal of Infectious Diseases: Vol.130, No.6: 673-676 (Dec) 1974.
Schimpff, S.C., Greene,W.H., Young, V.M., Fortner, C.L., Jepsen, L., Cusack, N., Block, J.B., and Wiernik, P.H.: Infection Prevention in Acute Nonlymphocytic Leukemia; Annals of Internal Medicine: Vol. 82: 351-358, 1975.

Keyword Descriptors: Clinical Research Pharmacy, Patient Oriented Pharmacy
Clinical Pharmacy

Project No. Z01 CM 06238-05 BCRC
1. BCRC
2. Clinical Research Pharmacy
Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Development of a Unit Dose Service and the Preparation of Parenteral Drugs

Previous Serial Number: 6238 and 6269

Prinicipal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove and Lawrence K. Westfall

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 3-½
Professional: 2
Other: 1-½

Project Description:

The objective is to improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labeled with a name, strength, and control number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. All of the parenteral products are prepared aseptically in a laminar air flow environment, and are dispensed in a clearly labeled ready to use form. Parenteral products are prepared for not more than a 24 hour period to ensure full potentcy. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patient's name. This information is also recorded by drug and lot number for each investigational agent.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed *the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct patient care and in general allows the hospital to demonstrate adequate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record. Physicians utilize these records daily in ordering medications for patients and have found them to be a useful and accurate source of information.*

During the past year the pharmacy service has dispensed approximately 250,824 oral doses of medication to inpatients, 24,938 I.V. admixtures, and 7,286 doses of parenteral investigational cancer chemotherapeutic agents.

By reducing medication errors, and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients care has increased. These delivery systems have been intergreted with the development of the Clinical Research Pharmacy Service which is providding the patients with better care. Thus, the research drug data will be improved.

Keyword Descriptors: Unit Dose, I.V. Additive, Cancer Chemotherapy

Project No. Z01 CM 06912-02 BCRC
1. BCRC
2. Clinical Research Pharmacy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System

Previous Serial Number: 6912

Principal Investigators: William R. Grove and Clarence L. Fortner

Other Investigators: Paul Chang, Robert Esterhay, George E. Nelson, Peter H. Wiernik

Cooperating Units: Medicine and Neurosurgical Service, BCRC; PROMIS Laboratory, Dept. Medicine, University of Vermont

Man Years:

Total: 1/16
Professional: 1/16
Other: 0

Project Description:

The objectives are to develop accurate, complete and concise antineoplastic drug displays for addition to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont. These displays provide easy access to referenced summarized pharmacological information which is retrievable at the touch-sensitive cathode ray screen terminal located at the nursing station at the BCRC.

Literature research is conducted to obtain accurate, complete, and up to date drug information on antineoplastic agents. This information is then consolidated to a specific format to conform with the over 20,000 displays currently on the PROMIS system. The format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, METABOLISM AND EXCRETION. All statements are fully documented to the specific reference source. Each completed display sequence is then sent to the PROMIS laboratory for auditing for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Updated pharmacologic information may be added to pre-existing displays by supplying this information to PROMIS laboratories for incorporation into the display sequences.

The antineoplastic drug displays summarize the current medical literature into concise, accurate, current, and complete displays of pharmacologic information of antineoplastic agents. The completed drug displays form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

This study will continue with additional antineoplastic drug displays being completed and entered into the computerized PROMIS system.

Keyword Descriptors: Pharmacology, Promis, Antineoplastics, Computer

Project No. Z01 CM 06937-01 BCRC
1. BCRC
2. Clinical Research Pharmacy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: A Patient Oriented Outpatient Drug Distribution Service

Previous Serial Number: None

Principal Investigator: Clarence L. Fortner

Other Investigator: William R. Grove

Cooperating Units: Medicine and Neurosurgical Services, BCRC

Project Description:

The objective of this service is not only to provide drugs, but to also provide drug information and drug administration instruction to outpatients and/or the patient being discharged from the hospital. These studies will include a method of best determining drug-drug interactions, drug-food interactions and known patient allergies prior to dispensing the drug to the patient. If this is the patient's first visit to the pharmacy, the patient will be questioned as to any new allergies and cautioned concerning drugs or foods to avoid if indicated. When the patient delivers the medication order to the pharmacy, the pharmacist reviews the order for completeness. The individual patient drug profile is obtained from the pharmacy file and the new drug ordered is reviewed against the previously prescribed drugs for drug drug interactions, drug-dietary interactions, and any known allergies. The outpatient drug order, at this point, will be prepared in the traditional method. The patient will be advised, at the time of dispensing, of common side effects and administration instruction. Patient compliances will be stressed by the pharmacist. The outpatient order for patients leaving the hospital will be compared with the patient inpatient drug record.

Preliminary findings would indicate that the patients are more aware, therefore, fewer patients administration error have been observed along with more patient compliance.

Keyword Descriptors: Outpatient Drug Order, Compliance, Patient Drug Profile Drug Interactions

SUMMARY REPORT

LABORATORY OF PHARMACOLOGY, BCRC, DCT, NCI

July 1, 1974, through June 30, 1975

Within the Enzymology and Drug Metabolism Section, concentration has been on gaining a greater understanding of the physiological role of polyamines in regulating cell metabolism. Previous work from the Section and elsewhere has shown a strong correlation between the presence of polyamines and RNA synthesis. Since the concentration of the RNA within the cell is not only a function of the amount synthesized, but also of the amount degraded, examination of the relationship between these substances and degradative enzymes (the ribonucleases) was undertaken.

Of particular interest tin this regard has been the observation that polyamines can cause not only an enhancement of RNase activity, but can also reverse the inhibition of RNase activity caused by certain polynucleotides. Thus polyguanylic and polyadenylic acids, which act as competitive inhibitors of RNase activity, are dissociated from the enzyme surface by spermidine. The significance of this is that it may help explain why the RNA in an RNA virus or in a messenger RNA is not readily degraded in mammalian cells. Both these RNAs have poly A at their 3'OH termini which can inhibit RNase activity. At the appropriate time the concentration of polyamine can rise causing reversal of the inhibition and subsequent degradation of the messenger RNA.

In view of the earlier findings that polyamines in human urine may have diagnostic importance in detecting neoplasia in man, the isolation of a protein (human plasma ribonuclease) from human plasma that can bind the polyamines is of considerable significance. The enzyme, although found in high concentrations in multiple myeloma, is related to that disease only in the sense that accompanying multiple myeloma is severe kidney disease. The enzyme concentration was found elevated in severe kidney malfunction unrelated to multiple myeloma.

Ongoing studies within the Section on specific ribonucleases and deoxyribonucleases have been of importance in the elucidation of the termini of an RNA isolated from AKR murine leukemia virus. Thus uridylic acid has been found at the 3' terminus, whereas adenylic acid is at the 5' terminus of the long chain polymer. Currently studies are being undertaken to examine the polarity of the viral RNA, particularly with respect to the section of the polymer coding for reverse transcriptase.

In collaboration with the Oncology Service of the Baltimore City Hospitals, studies on the use of polyamine levels in human urine as an indicator of the presence of neoplasia are ongoing. Thus far from the results obtained with patients undergoing extensive chemotherapy, what appears to be of particular significance in evaluating the disease's state is the concentration of spermidine.

The Section of Immunology and Cell Biology has continued work utilizing immunologically induced in vitro lymphocyte responsiveness as a model of human lymphocyte reactivity to non-self. Important advances have been made which relate both generally to the evaluation of host immune responsiveness, the viral infectious disease area and more specifically, to applications of this assay for evaluation of lymphocyte reactivity to putative oncogenic viruses. This laboratory has presented evidence that purified (banded) rubeola virus is not capable of eliciting responsiveness in this system but that responsiveness can be observed when the cell membrane preparation from which the virus is purified is used an antigen. This finding is of importance since it points to the need for membrane associated antigen as the recognition template (either viral or cell) in order for lymphocyte responsiveness to be achieved. Application of this observation to other viruses has indicated that a viral infected cell membrane preparation is generally the best antigenic source to determine previous host exposure to virus in the lymphocyte blastogenesis system. Studies in this laboratory have also focused on defining the potential and mechanism of action of various immune adjuvants in the prevention and treatment of neoplasia. The effectiveness of polyadenylic polyuridylic acid in the prevention of AKR leukemia has been re-explored and an age dependent effect was noted. It has been defined that the beneficial effect of polyadenylic polyuridylic acid in prolonging survival and preventing leukemia in the AKR mouse was dependent upon giving this agent at an early age rather than late. Additional work has explored the generally accepted concept that immunotherapy is most effective when tumor burden is at its lowest point. It has been found that immunotherapy initiated at a point of low tumor burden may result in some situations in decreased survival and that increased survival is observed if treatment is delayed. This work is of importance because it brings to the forefront the concept that tumor antigenic concentration must be sufficient for the host to respond successfully to tumor. Because of immune adjuvant work suggesting that abnormal ribonuclease activity existed in mice with neoplasia a series of studies have been carried out reflecting that abnormal nucleolytic activity is capable of predicting the development of tumor in high incidence tumor strain mice. Similarly, elevation of nuclease activity was found in the serum of white cells of mice with transplantable leukemia, decreased depression of nuclease activity in the white cells of guinea pigs bearing L2C leukemia and an elevation of serum nuclease activity in patients with neoplasia.

Studies concerning the interaction of tumor specific antibody and tumor and normal cells has indicated that the presence of an active homologous complement source capable of mediating tumor specific antibody lysis of tumor cells is also capable of mediating significant cross reactivity to antigenic determinants on normal cells. This cross reactivity is not observed when this same antibody is absorbed in the absence of an active complement source.

Efforts in the Biochemistry Section have been directed in a broad program of basic and clinical research of cancer chemotherapeutic agents as well as in the study of the controlling factors of cell growth and differentiation. By comparing the pharmacologic disposition of adriamycin in man and in the rabbit, we have isolated and identified several new metabolites. In our rabbit studies we identified adriamycinol as the major metabolite of adriamycin. A cytoplasmic enzyme is responsible for its formation which requires TPNH as cofactor. This parallels what we originally described for the daunorubicin-daunorubicinol relationship. From our comparative studies, we feel that the anthracycline antibiotic induced cardiotoxicity is related to a specific sensitivity of the heart rather than a unique handling of the anthracyclines by man and rabbit.

In our study of the human metabolism of daunorubicin, we identified demethyl deoxydaunorubicinol aglycone, deoxydaunorubicinol aglycone 13-O-β glucuronide, demethyl deoxydaunorubicinol aglycone 4-O-sulfate, and demethyl deoxydaunorubicinol aglycone 4-O-β glucuronide as new human metabolites. Several other metabolites were isolated but not identified. From these studies combined with our enzyme studies, we have proposed a new metabolic pathway for this agent.

By closely following the adriamycin turnover in a patient with an indwelling T-tube, we have obtained the best estimate yet obtained of the human disposition of this drug. The cumulative biliary excretion accounts for 40% of the administered drug with about a 13% output in the urine. Several metabolites were identified and quantified. This study substantiates our prior indirect evidence that the biliary route is the primary pathway in man for excretion of adriamycin.

Our studies on endogenous tissue growth substances have allowed us to partially purify a hepatic regenerative stimulator substance and to characterize physiologic parameters of its activity in normal animals. Several chemical characteristics of this substance were determined.

We have developed affinity absorption purification techniques for the isolation of phytohemagglutinin (PHA) lectins. Through this technique we are able to prepare active components of the PHA family in quantities and purity necessary for detailed membrane specificity studies in normal and malignant cells.

Project No. Z01 CM 06012-05 BCRC
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Biochemical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6012

Principal Investigator: Nicholas R. Bachur

Other Investigators: Ronald Felsted
Hiroaki Kubo
Marta Kaber
Malcolm Gee

Cooperating Units: None

Man Years (July 1, 1974 to June 30, 1975)

Total:	3.3
Professional:	1.3
Other:	2.0

Project Description:

To study the biochemical pharmacology of selected cancer chemotherapeutic agents and to provide a scientific foundation for their usage. To resolve the metabolic pathways of cancer chemotherapeutic agents and to study the interactions of these drugs and their metabolites with tissue components *in vitro* and with organized physiological systems *in vivo*.

We studied the human metabolism of daunorubicin by isolating and identifying metabolites which retained the specific fluorescence properties of daunorubicin. Metabolites were extracted by absorption to polystyrene polymeric sorbants, separated on silicic acid columns, and purified by thin layer chromatography. Structures were determined by comparative chromatography; infrared, fluorescence, and mass spectroscopy; and enzymatic and chemical degradation. Daunorubicin metabolites identified are daunorubicinol, daunorubicinol aglycone, deoxydaunorubicin aglycone, deoxydaunorubicinol aglycone, demethyl deoxydaunorubicinol aglycone, deoxydaunorubicinol aglycone 13-O-β glucuronide, demethyl deoxydaunorubicinol aglycone 4-O-sulfate, and demethyl deoxydaunorubicinol aglycone 4-O-β glucuronide. Other metabolites have been purified but not identified. Human metabolism of daunorubicin involved carbonyl reduction, reductive glycosidic cleavage, O-demethylation, O-sulfation, and O-glucuronidation. Since daunorubicinol has biochemical properties similar to daunorubicin, the metabolites may contribute to the pharmacologic and toxicologic effects credited to daunorubicin.

The understanding of the biochemical alterations that active chemotherapeutic agents undergo is central to the understanding of their usage. This is of importance with the anthracycline antibiotics since we have observed a rather extensive metabolism of both adriamycin and daunorubicin *in vivo* and *in vitro*.

Study of the comparative metabolism of daunorubicin, adriamycin, and other anthracycline antibiotics in animals and in humans will be carried out. The isolated metabolites of adriamycin and daunorubicin are being assessed for their pharmacologic and toxicologic activity so that we can obtain an overall understanding of their structure-activity relationships.

Keyword Descriptors: daunorubicin, daunorubicinol, daunorubicinol aglycone, deoxydaunorubicin aglycone, deoxydaunorubicinol aglycone, demethyl deoxydaunorubicinol aglycone, deoxydaunorubicinol aglycone 13-O-β glucuronide, demethyl deoxydaunorubicinol aglycone 4-O-sulfate, demethyl deoxydaunorubicinol aglycone 4-O-β glucuronide, carbonyl reduction, reductive glycosidic cleavage, O-demethylation, O-sulfation, O-glucuronidation

Publications: Egorin, M.J., Hildebrand, R.C., Cimino, E.F., and Bachur, N.R.: Cytofluorescence localization of adriamycin and daunorubicin. *Cancer Res.* 34: 2243-2245, 1974.

Project No. Z01 CM 06014-05 BCRC
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation

Previous Serial Number: NCI-6014

Principal Investigators: Nicholas R. Bachur
Ronald Felsted
Richard D. Leavitt

Cooperating Units: None

Man Years (July 1, 1974 to June 30, 1975)

Total:	1.7
Professional:	1.7
Other:	0

Project Description:

To study the control mechanisms which affect cellular growth and differentiation. These studies are aimed at elucidating control principles effecting all levels of cellular machinery as well as various levels of physiological control of the organized functioning animal. To determine differences in controls between differentiated and nondifferentiated tissues and between malignant and normal tissues. To studv the effects of control changes on physiologic processes.

The elucidation of the biochemical controls of growth processes is basic to understanding both normal and malignant growth. The exquisitely sensitive control demonstrated in the regenerating liver provides an excellent model system for growth studies. Intraperitoneal injection of a weanling rat liver extract into 34% hepatectomized rats stimulates ^{3}H thymidine incorporation into recipient hepatic DNA 2.5 fold. This stimulation is seen after a 10 hour lag period. Extracts from weanling rat liver contain the highest level of the hepatic regenerative stimulator substance, whereas extracts from adult livers give no significant stimulation. However, if adult rats are partially hepatectomized, hepatic regenerative stimulator substance is demonstrated in their livers 12-15 hours post-hepatectomy. Diurnal rhythm of the hepatic regenerative stimulator substance is observed in weanling rats with peak activity at 3-9 A.M. and nadir at 6-9 P.M. Partial characterization of hepatic regenerative stimulator substance shows that it is a soluble material not sedimented by centrifugation for 2 hours at 145,000 xg. The stimulator activity is stable to heating at 60°C and 100°C for 15 minutes but is lost when treated with 10% perchloric acid. Activity is not lost following membrane dialysis and the hepatic regenerative stimulator substance has a molecular weight of about 10,000.

In our studies on the cell-cell interaction and the mechanism of action of agglutinating substances, we are developing new preparative techniques to prepare purified lectins. Half-gram quantities of phytohemagglutinin lectins are purified from saline extracts of red kidney beans (Phaseolus vulgaris) by affinity absorption on porcine thyroglobulin-Sepharose. Polyacrylamide gel electrophoresis of the isolated fraction reveal a mixture of five distinct proteins. Although all of the five proteins specifically bind to porcine thyroglobulin, the cathodal migrating proteins bind more strongly than the anodal migrating proteins. Studies with protein mixtures relatively depleted of different components identify the most cathodal and anodal migrating proteins, respectively, as the erythrocyte and lymphocyte specific components of red kidney bean phytohemagglutinin. Exhaustive absorption of saline bean extracts removes all of the mitogenic and erythroagglutinin activity. The erythroagglutinin activity of the purified mixture is enriched 24.6 fold over saline extract activity and represents a 73.5% recovery. This simple, efficient method is used to prepare all the active components of the phytohemagglutinin family in the quantities and purity necessary for further studies.

The isolation of materials from both plant and mammalian sources which effect the growth and differentiation of normal lymphocytes and hepatocytes is paramount in the study of the processes which control cellular activities. By using these purified materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways are possible, we are approaching techniques where chemical agents may be used to control cellular growth, differentiation, and proliferation.

We will continue our studies on the control mechanisms which affect cellular growth and differentiation, and attempt to elucidate control principles affecting all levels of cellular machinery. Specifically, we hope to further purify our hepatic stimulator substance and to describe its physiological action in the normal animal. We hope to elucidate the mechanism of action of the isomitogens on cell membranes and determine how these materials cause mammalian cells to adhere to surfaces. Thirdly, we will continue our search for materials characteristic of altered metabolic pathways, which may be used to quantify malignant cells in vivo.

Keyword Descriptors: regenerating liver, hepatic regenerative stimulator substance, phytohemagglutinin, affinity absorption, mitogenic, erythroagglutinin

Publications: Puri, S., Belliveau, R.E., Spencer, R.P., and Bachur, N.R.: Prostaglandins in mice with neuroblastoma. J. Nucl. Med. 16: 83-85, 1975.

Project No. Z01 CM 06016-05 BCRC
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Pharmacodynamics of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6016

Principal Investigator: Nicholas R. Bachur

Other Investigators: M. Steele
R. S. Jaenke

Cooperating Units: College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado

Man Years (July 1, 1974 to June 30, 1975)

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

To investigate the biochemical and physiological effects of anticancer drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Rabbits, animals which develop a cardiotoxicity from adriamycin and daunorubicin, excrete about 17% of intravenously injected adriamycin or daunorubicin via their bile and only 2% of the drugs in their urine for 8 hours after administration as measured by drug fluorescence. Thin-layer analysis of bile and urine indicates extensive metabolism of adriamycin and daunorubicin, principally the carbonyl reduction of both, to adriamycinol and daunorubicinol, respectively. Generally daunorubicin is metabolized more than adriamycin. Other polar metabolites, presumably conjugates, are the next most prevalent group of metabolites. Tissues contain higher levels of daunorubicin and adriamycin and substantial quantities of reduced metabolites of both. Free aglycone metabolites are not prominent except in liver, kidney, and small intestine. No unusual metabolite or high drug or metabolite levels are found in the heart to account for the cardiotoxicity. Homogenates and soluble fractions of rabbit liver and kidney metabolize both adriamycin to adriamycinol and daunorubicin to daunorubicinol with a dependence on reduced triphosphopyridine nucleotide. Isolated adriamycinol was identified by comparative thin-layer chromatography and ultraviolet-visible and mass spectroscopy.

Adriamycin and daunorubicin are metabolized extensively *in vivo* and *in vitro* by rabbits. Both antibiotics are reduced respectively to glycol or alcohol glycosides by a reductase(s) that is cytoplasmic in location. Although other investigators examined the *in vivo* disposition of adriamycin, the metabolic transformation of adriamycin to the glycol adriamycinol was not reported. Carbonyl reduction is the major *in vivo* metabolic conversion for the anthracycline antibiotics in the rabbit and results in substantial levels of the reduced metabolites in tissues, bile, and urine. Our *in vivo* and *in vitro* data indicate that daunorubicin is more readily reduced than adriamycin. This agrees with our studies on the substrate specificity of the purified rat liver reductase which reduces daunorubicin about 20 times the rate of adriamycin reduction although the K_m for both substrates are quite similar. The differential enzyme specificity for substrate is reflected in the levels of adriamycinol and daunorubicinol found in tissues, bile, and urine.

Among the other animals we examined for *in vivo* metabolism of adriamycin, the human resembles the rabbit in the degree of conversion of adriamycin to adriamycinol. At present, we do not know whether this similarity is related to the level of reductase in rabbit and human tissues or to the specificity of the reductase for adriamycin.

Although the rabbit has an unusual cardiac sensitivity to the anthracyclines, we see no apparent reason for this from these acute drug disposition studies. No unusual fluorescent metabolites are detected in the rabbit heart. Although other investigators reported that aglycones of adriamycin and daunorubicin are elevated in cardiac tissue and are associated with cardiotoxicity in hamsters, the aglycone levels in the rabbit heart are not unusual and are, in fact, significantly lower than the aglycone concentrations reported by those authors. The aglycone concentrations measured in our study in rabbit hearts are no greater than aglycone levels we have determined in similarly treated hamsters and rats. Concentrations of adriamycin and daunorubicin in heart are not excessive or unusually high. Unless some undetected drug form is related to the cardiopathology, it appears that the anthracycline-associated cardiotoxicity in the rabbit is more likely related to a specific sensitivity of the heart rather than a unique handling of the anthracyclines.

Since adriamycinol, like daunorubicinol, is not modified in a fashion that destroys its biochemical efficacy as an inhibitor of nucleic acid synthesis in cells, the metabolism of adriamycin to adriamycinol may contribute to the action and toxicity of this drug. Adriamycinol, although it is more polar than the parent drug, retains ability to inhibit nucleic acid synthesis in cultured mouse leukemia cells. The abundant levels of adriamycinol that we observed in the urine and plasma of patients treated with adriamycin emphasize this possibility.

Through the continued investigation of the pharmacodynamics of drugs we may be able to determine why these agents possess differences in their therapeutic index and spectrum of action.

NCI-06016

Keyword Descriptors: rabbits, cardiotoxicity, adriamycin, daunorubicin, adriamycinol, daunorubicinol, carbonyl reduction

Publications: Bachur, N.R., Hildebrand, R.C., and Jaenke, R.S.: Adriamycin and daunorubicin disposition in the rabbit. J. Pharmacol. Exp. Ther. 191: 331-340, 1974.

Project No. Z01 CM 06241-05 BCRC
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Clinical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6241

Principal Investigator: Nicholas R. Bachur

Other Investigators: Steven Reich
Charles Riggs Jr.
Peter H. Wiernik

Cooperating Units: Medicine Service, Baltimore Cancer Research Center, NCI

Man Years (July 1, 1974 to June 30, 1975)

Total:	1.5
Professional:	1.3
Other:	0.2

Project Description:

To study the disposition of cancer chemotherapeutic agents in humans with respect to absorption, distribution, biotransformation, and excretion. To characterize drug effects, potency, and maximum efficacy. To evaluate the toxicity of cancer chemotherapeutic agents in man and observe aspects of biological variations and other factors such as age, body weight, sex, route of administration, time of administration, rate of inactivation and excretion, tolerance, pathological state, genetic factors, and drug-drug interaction. To devise appropriate dosage schedules. To develop ancillary therapy to minimize toxic effects of drugs without effecting the therapeutic value.

Because of the importance of biliary excretion of adriamycin in experimental animals and indirect evidence for its importance in man from samples of autopsy bile and abnormal plasma pharmacokinetics in patients with liver disease, this study was designed to quantify human biliary excretion of adriamycin. Bile from an indwelling T-tube as well as plasma and urine were collected over a one week period from a patient with histiocytic lymphoma receiving adriamycin therapy. His liver function and plasma pharmacokinetics were normal at the time of study. Samples were extracted with chloroform: isopropanol (1:1), separated by thin-layer chromatography, and analyzed fluorometrically for adriamycin and metabolites. Cumulative biliary excretion of adriamycin and fluorescent metabolites accounted for 40% of the administered dose compared with 13% for urinary excretion. The most prominent biliary species was adriamycin followed by adriamycinol, accounting for 17% and 9% of the administered dose respectively. Four conjugated metabolites, accounted for 7%, 4 aglycones for 5%, and 2 less polar metabolites for 3%. This study substantiates our prior indirect evidence that the biliary route is the primary pathway in man for excretion of adriamycin and its fluorescent metabolites and correlates with increased toxicity in patients with liver disease. Incomplete drug recovery suggests prolonged tissue retention and/or conversion to non-fluorescente metabolites.

These studies have established and correlated clinical and pharmacologic observations for the scientific usage of adriamycin in malignant disease.

We will continue to study the clinical pharmacology of cancer chemotherapeutic agents and attempt to related fundamental laboratory findings to clinical observations.

Keyword Descriptors: biliary excretion, adriamycin, abnormal plasma pharmacokinetics, liver disease, conjugated metabolites

Publications: Bachur, N.R.: Adriamycin-daunorubicin cellular pharmacodynamics-Symposium on Clinical Pharmacology. Biochem. Pharmacol. 23: Supp. 2, 207-216, 1974.

Benjamin, R.S., Wiernik, P.H., and Bachur, N.R.: Adriamycin: A new effective agent in the therapy of disseminated sarcomas. Med. and Ped. Oncol. 1: 63-76, 1975.

Project No. ZO1 CM 06210 05 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Development of Specific RNases as an Aid in Sequencing of tRNA from Murine Leukemia Viruses

Previous Serial Number: NCI-6210

Principal Investigator: C. C. Levy

Man Years (July 1, 1974, through June 30, 1975

Total	:	1/2
Professional	:	1/2
Other	:	0

Project Description

The purpose of the study was the isolation from microbial sources of nucleases which will hydrolyze long chain nucleic acids at specific base linkages as an aide in the sequential analysis of RNA from various murine leukemia viruses.

The technique of enrichment culture was employed to isolate a number of microorganisms from soil which could degrade RNA at specific nucleotide bonds. Thus far nucleases specific for bonds containing cytidylic acid and uridylic acid have been obtained from two of the organisms. A third, Serratia sp., elaborates an enzyme which shows preferential specificity for adenylic acid linkages. The second of the two organisms described, Citrobacter sp., which hydrolyzes uridylic acid internucleotide bonds, has shown interesting alterations in its specificity under the influence of the polyamine, spermidine. The Citrobacter nuclease shows no activity towards polycytidylic acid until spermidine is introduced into the reaction mixture. At this point the degradation of cytidylic acid containing nucleotide bonds is rapid and complete. Of considerable interest is that this is the first instance in which spermidine has been shown to have a direct effect on an enzyme. Of all the polyamines tested, the enzyme binds spermidine preferentially. The binding of the polyamine to the enzyme is the first case known where spermidine is bound to a protein. This binding has enabled us to isolate the peptide region of the enzyme to which the polyamine is bound. Two peptides have been isolated and the amino acid composition of both have been determined. It has been shown that for every mole of spermidine bound, one mole of peptide can be isolated.

Nucleic acid metabolism or, more correctly, the alteration of nucleic acid metabolism, is believed to play an important role in cancer induction. It is hoped that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer in tumor-causing viruses may provide a clue to the nature of cancer induction. In addition, the effect of spermidine on the enzyme which hydrolyzes polyuridylic acid is of considerable interest. The reason is that in the urine of patients with diagnosed cancer there appears to be an increase in polyamine levels. The relationship of spermidine to the Citrobacter enzyme may have significance in terms of the physiological changes which are occurring in the cell as the tumor progresses. In addition, the ability of the protein to bind polyamines suggests that nucleases may play a considerably more pivotal role in the regulation of the cellular metabolism than had hitherto been suspected. This type of phenomena will be investigated in human enzymes as well, particularly in the enzymes that may be found in patients with cancer.

The projected course of this study is to utilize these enzymes as a means of sequencing RNA isolated from tumor viruses. It is hoped that by establishing partial sequences in a number of RNAs from various tumor viruses, some relationship between the sequence and neoplasia will be seen.

Keyword Descriptors

Polyamine effects, specific RNases, human plasma ribonuclease, RNA sequencing studies.

Publications

Friedling, S. P., Schmukler, M. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. I. Purification and general properties. Biochim. Biophys. Acta 268: 391-402, 1972.

Schmukler, M., Friedling, S. P. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. II. Studies with synthetic polyribonucletides. Biochim. Biophys. Acta 268: 403-410, 1972.

Levy, C. C., Mitch, W. E. and Schmukler, M. Effect of polyamines on a ribonuclease which hydrolyzes RNA at uridylic acid residues. J. Biol. Chem. 248: 5712-5719, 1973.

Marotta, C. A., Levy, C. C., Weissman, S. M. and Varricchio, F. Preferred sites of digestion of a ribonuclease from Enterobacter in the sequence analysis of Bacillus stearothermophilus 5S ribonucleic acid. Biochemistry 12: 2901-2904, 1973.

Levy, C. C., Mitch, W. E. and Schmukler, M. Influences of polyamines on the hydrolysis of polynucleotides by Citrobacter nuclease. In Polyamines in Normal and Neoplastic Growth (Ed. D. H. Russell) Raven Press, New York, 1973, pp. 91-101.

Levy, C. C., Hieter, P. A. and LeGendre, S. M. Evidence for the direct binding of polyamines to a ribonuclease that hydrolyzes ribonucleic acid at uridylic acid residues. J. Biol. Chem. 249: 6762-6769, 1974.

Schmukler, M., Jewett, P. B., and Levy, C.C. The effects of polyamines on a residue-specific human plasma ribonuclease. J. Biol. Chem. 250: 2206-2212, 1975.

Project No. ZO1 CM 06242-05 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title	:	Polyamines and Cancer; Functional Significance
Previous Serial Number:		NCI-6242
Principal Investigator:		T. P. Karpetsky
Other Investigators	:	C. C. Levy
Cooperating Units	:	Baltimore City Hospitals, Oncology Department
Man Years		(July 1, 1974, through June 30, 1975)
Total	:	0.1
Professional	:	0.1
Other	:	0

Project Description

In order to define the functions of putrescine, spermidine, and spermine and to use this information in cancer diagnosis and control, the levels of these compounds in the urine of AML and ALL patients were determined by use of a modified amino acid analyzer. Correlation of levels of individual polyamines with state of disease and course of treatment was determined from patient medical records.

Initial studies indicated that the polyamines are elevated in patients with cancer. However, putrescine levels appear to reflect both chemotherapy and state of disease. Spermidine levels accurately reflect the state of disease, being elevated above normal control values prior to treatment, then failling to near normal values as remission of disease is obtained. Spermine levels are always very low and these measurements are not useful indicators of state of disease or course of treatment. Measurements of overall polyamine levels are misleading since the level of putrescine can change drastically when antibiotics are administered.

There is, therefore, evidence to indicate that the levels of spermidine may be used as a clinical tool for initial diagnosis of cancer patients and as an indicator showing when remission has been obtained. Moreover, the interpretation of total polyamine levels and, in particular, putrescine levels, is open to question.

Recent acquisition of a polyamine analyzer (Technicon) will facilitate the handling of larger numbers of samples and it is hoped that serum as well as urine may be examined. Furthermore, by different sample handling techniques, free versus bound polyamines may be investigated to determine which of these levels is more significant as a biomarker for disease. It is planned to do further studies involving regular screening of AML and ALL patients with expansion into areas where remission is less marked or takes a longer time course (e.g., solid tumors of various types). Also correlations of polyamine levels with level of serum ribonuclease (RNase) in patients with multiple myeloma or severe renal disease will be attempted. Finally, studies of polyamine levels in states of rapidly proliferating human tissue; e.g., pregnancy, massive hepatic damage, etc., will be undertaken. These results will be compared with levels obtained from cancer patients with the aim of identifying possible false positives in the spermidine level test for cancer diagnosis.

Keyword Descriptors

Polyamines, polyamine analysis, cancer diagnosis, biomarkers, ribonuclease.

Publications

None.

Project No. Z01 CM 06247-05 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title :	Purification and Study of Bacterial and Mammalian Nucleases with Differing Residue Specificities
Previous Serial Number:	NCI-6247
Principal Investigator:	M. Schmukler
Other Investigators :	C. C. Levy, W. Drake, M. Mardiney
Cooperating Units :	None
Man Years	(July 1, 1974, through June 30, 1975)
Total :	1 1/2
Professional :	3/4
Other :	1/2

Project Description

These studies of nucleases are conducted in order to find enzymes suitable for sequencing nucleic acids, to study enzymatic control mechanisms, and to evaluate nuclease levels as indicators of the presence of malignant disease.

A human plasma ribonuclease purified more than 2000-fold is potently inhibited by synthetic polyribonucleotides such as poly G, poly X, poly A, poly (A,G), poly (G,I), and poly (G,U). Low concentrations of the polyamines, spermine and spermidine, are able to reverse these inhibitions. These polyamines also cause a reduction in the molecular weight of the enzyme. An inhibitor of this enzyme has been found in human tissues, and its effect is also reversed by polyamines. Plasma ribonuclease activities are elevated in 70% of individuals with malignant disease.

An understanding of nucleic acid metabolism should be of help in the detection and control of malignant diseases since most treatments of cancer involve an alteration of nucleic acid metabolism. Some of the enzymes and inhibitors studied may be useful ultimately in chemotherapy.

Keyword Descriptors

Plasma ribonuclease, inhibitors, polyamines, spermine, spermidine, control mechanisms, molecular weight, polyribonucleotides, inhibition reversal.

Publications

Schmukler, M., Jewett, P., and Levy, C. C. The effects of polyamines on a residue-specific human plasma ribonuclease. J. Biol. Chem. 250: 2206-2212, 1975.

Project No. Z01 CM 06902-04 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Characterization of Viral RNA Isolated from AKR Murine Leukemia Virus

Previous Serial Number: NCI-6902

Principal Investigator: J. J. Frank

Other Investigators : C. C. Levy

Man Years (July 1, 1974, through June 30, 1975)

Total : 1/4
Professional : 1/4
Other : 0

Project Description

The purpose of the study was the isolation of undegraded AKR murine leukemia viral RNA for the purpose of determining the identity of the 3' and 5' termini. Further work would include the determination of the polarity with which a message contained in the RNA is read on the mouse ribosome.

The viral RNA has been isolated using a technique in which the virus is disrupted by a combination of phenol, sodium dodecal sulfate and salt solution. The viral RNA is extracted into the aqueous phase and separated from lower molecular weight RNA by glycerol gradient centrifugation. The viral RNA was then subjected to alkaline phosphatase treatment. The phosphate at the 3' and 5' termini are removed and the RNA at the 5' end phosphorylated with polynucleotide kinase and ATP^{32}. The 5' terminus of the RNA is then released by digestion with alkali to give the 3'-5' nucleotide diphosphates. Analysis by paper chromatography and high voltage electrophoresis against standards made from the 3' nucleotide phosphates has shown that adenylic acid is the nucleotidy at the 5' end of the RNA. The 3' terminus was identified after alkaline phosphatase treatment by oxidation of the terminal sugar residue to the dialdehyde followed by reduction to the trialcohol with tritiated $NaBH_4$. Two-dimensional thin layer chromatography of the alkaline digest of labeled RNA indicated the 3' terminus was uridylic acid. The viral RNA has also been hydrolyzed lyzed with base specific ribonuclease. Preliminary studies have shown that one enzyme can degrade viral RNA into oligonucleotides with chain links approximately 10-20 nucleotides in length.

An understanding of the primary sequence of the viral RNA should be helpful in the elucidation of the mechanism by which the virus may replicate itself. Thus if that portion of the viral RNA responsible for the enzyme "reverse transcriptase" is known, then it may be possible to design drugs which can destroy it.

It is proposed to continue the analytical studies and establish the polarity by which the RNA is read on the mouse ribosome.

Keyword Descriptors

AKR viral RNA, termini, polynuclotide kinase, NaB^3H_4.

Publications

Frank, J. J., Dorr, R. G., and Levy, C. C. Characterization of the 5'-terminus of murine leukemia virus RNA. Biochim. Biophys. Acta 366: 353-357 (1974).

Project No. Z01 CM 06903-03 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Development of Specific DNases as an Aid in the Sequencing of DNA from Various Viral Tumors

Previous Serial Number: NCI-6903

Principal Investigator: C. C. Levy

Man Years (July 1, 1974, through June 30, 1975)

Total : 1/2
Professional : 1/2
Other : 0

Project Description

The purpose of the study was the isolation from microbial sources of a DNase which will hydrolyze deoxyribonucleic acid at a specific base linkage as an aid in the sequential analysis of DNA obtained from a tumor virus.

The technique of enrichment culture was employed to isolate DNases from a microorganism which degrades DNA at specific nucleotide linkages. The organism has been classified as a Flavobacter and the enzyme has been characterized to some degree. The enzyme has a molecular weight of at least 200,000 and presumably breaks up into four, perhaps six, subunits. The enzyme appears to be an endonuclease in that alteration of the termini of various substrates has no effect on enzyme activity. The enzyme has strong specificity for deoxyadenylic acid, since it will not attack ribonucleic acid or polyribonucleotides under any conditions. The enzyme should be helpful in elucidating the primary sequence of DNA from tumor viruses. The enzyme, purified 2000-fold, will bind the polyamines, particularly spermidine. The binding site on which the polyamine adheres to the protein has been found to be a nucleophilic center. Thus this finding indicates that not only RNases but also DNases can bind polyamines. The binding site, at least with respect to the amino acid composition, appears to be generally similar. The significance of this finding is currently being explored.

The ability to degrade deoxyribonucleic acid at specific linkages when the nucleic acid is obtained from viral sources should be of intense interest in sequencing the DNA of a tumor virus. It is hoped to compare regions of the DNA with comparable areas isolated from RNA tumor viruses.

It is proposed to isolate a homogeneous DNA from viral sources and to attempt a sequential analysis of the nucleotide composition. An attempt will be made to sequence the peptide isolated which binds spermidine and see the significance of this binding site with respect to the binding site on the ribonucleases. Since at the same site large ordered polynucleotides (poly AU) bind to the protein, it is of interest to determine if this binding site is the same and, if the same, is this a general phenomena throughout biological proteins? Would, for example, the binding site to which poly AU adheres be the same on a membrane surface, a surface which is of considerable importance in human chemistry?

Keyword Descriptors

Specific DNases, DNA sequences, DNA viruses.

Publications

Frank, J. J., Hawk, I. A., and Levy, C. C. Polyamine activation of staphylococcal nuclease. Biochim. Biophys. Acta (in press) 1975.

Project No. ZO1 CM 06925-02 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Studies on the Metabolism, Function, and Storage of Human Blood Platelets

Previous Serial Number: NCI-6925

Principal Investigator: M. Schmukler

Other Investigators : P. D. Zieve

Cooperating Units : Department of Medicine, Baltimore City Hospitals

Man Years (July 1, 1974, through June 30, 1975)

Total : 1 1/4
Professional : 3/4
Other : 1/2

Project Description

These studies are designed to elucidate metabolic, pharmacologic, and storage effects upon platelet function and viability. Information obtained from these studies should be helpful in leading to more effective use of platelet transfusions in control of bleeding in cancer patients receiving chemotherapy.

Studies of the interaction of con A and thrombin upon the platelet release reaction suggest the presence of at least two types of release receptors on the platelet membrane: 1) those causing release of serotonin, nucleotides and αmannosidase; 2) and those associated with release of other glycosidases. The fact that con A inhibits thrombin-induced release of glycosidases and thrombin-induced aggregation suggests that there is a competition between these compounds for the same receptors.

Further studies demonstrated the ability of platelets to incorporate glucosamine and mannose into protein. This incorporation is reduced by thrombin, apparently through hydrolysis of glycoproteins, presumably on the platelet membrane.

Collection of granulocytes and platelets from donors is facilitated by the use of hydroxyethyl starch. We have shown that the latter compound does not adversely affect platelet function in vitro and hence is satisfactory for use in cell separators.

Studies with con A and thrombin, and studies of con A binding to platelets will be pursued further.

Keyword Descriptors

Concanavalin A, thrombin, aggregation, release reaction, membrane receptors, platelet storage, platelet transfusion, serotonin, nucleotides, glycosidases, glycoprotein, glucosamine, mannose, hydroxyethyl starch.

Publications

Schmukler, M., and Zieve, P. D. The effect of concanavalin A on human platelets and their response to thrombin. J. Lab. Clin. Med. 83: 887-895, 1974.

Zieve, P. D. and Schmukler, M. Incorporation of carbohydrate into protein of human platelets. Biochim. Biophys. Acta 372: 225-229, 1974.

Schiffer, C. A., Aisner, J., Schmukler, M., Whitaker, C. L., and Wolff, J. H. The effect of hydroxyethyl starch on in vitro platelet and granulocyte function. Transfusion, in press.

Project No. ZO1 CM 06926-02 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Role of Polyamines in Regulating Activity and Specificity of Various Nucleases

Previous Serial Number: NCI-6926

Principal Investigator: T. P. Karpetsky

Other Investigators : C. C. Levy

Man Years (July 1, 1974, through June 30, 1975)

Total : 0.3
Professional : 0.3
Other : 0

Project Description:

In order to establish the mechanisms by which the polyamines, putrescine, spermidine, and spermine regulate the enzymatic activity and substrate specificity of ribonucleases (RNases), a number of different biochemical systems were examined. For example, spermidine reverses enzymatic inhibition caused by certain metals. Spermidine was more effective in this respect than lower homologs and this effect is not due to simple chelation and removal of metal ions. Furthermore, spermidine was found to reverse inhibition of an RNase from Citrobacter sp. caused by an endogenous inhibitor. This system served as a model for the investigation of the effects of spermidine reversal of the inhibition caused by the mRNA fragment, poly A. Spermidine can, therefore, act as a concentration-dependent regulator of enzymatic activity when these inhibitors are present. Furthermore, since the polyamines and several polyamine analogs can greatly extend the half-life of RNases when subjected to thermal denaturation (37°), these compounds act as regulators of enzyme amount over time as well. In order to establish the minimum structural features required for polyamine activity in the reversal of denaturation, many analogs of spermidine were tested. It was found that a reduction of one carbon in either branch of spermidine results in retention of activity. Also a nitrogen at a terminal position is not required, oxygen in certain compounds functioning just as well as nitrogen. The conclusions drawn were that the spermidine-like linkage must be available for presentation on a surface along the "edge" of a molecule and must not be buried in the "center" of a large molecule. Furthermore, it is the spacing of heteroatoms that is crucial in conjunction with the partial charge on these heteroatoms. Studies are continuing in efforts to understand the regulatory role of spermidine and other polyamines by examination of these compounds in other nuclease systems. Further work is being conducted on the structural requirements for polyamine activity by the testing of other analogs. The possibility of different spermidine receptors for different enzymatic functions (enhancement of activity, protection from denaturation, etc.) is also being explored using polyamine analogs.

Keyword Descriptors

Polyamines, ribonucleases, denaturation, analogs, inhibition, regulation.

Publications

None

Project No. Z01 CM 06927-02 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title :	Properties of the *Enterobacter* Nuclease; Interaction of Nucleases with the Polyamines
Previous Serial Number:	NCI-6927
Principal Investigator:	J. J. Frank
Other Investigators :	C. C. Levy
Man Years	(July 1, 1974, through June 30, 1975)
Total :	1/2
Professional :	1/2
Other :	0

Project Description

The purpose was to isolate a nuclease which can hydrolyze phosphodiester bonds at particular residues in RNA and would therefore be useful in determining the primary sequence of various types of ribonucleic acid. Further, it was of interest to determine the interaction of nucleases with the polyamines which have been shown to bind to and affect the activity of *Citrobacter* nuclease.

The C ribonuclease was isolated from *Enterobacter* sp. which had been obtained from soil, using enrichment culture techniques. After preliminary purification the enzyme was subjected to DEAE-cellulose chromatography followed by either a heat step or a putrescine affinity column. The C ribonuclease was homogeneous and had a molecular weight of 26,000. The nuclease was shown to digest only poly C and not poly A, poly U or poly G. Digestion of RNA indicated that the C ribonuclease cleaves between the 3'-phosphoryl moiety of cytidylic acid and the 5'-hydroxyl group of adenylic acid. The pH maximum for enzyme activity in either Tris-maleate or Tris-HCl buffers was between 7.0 and 8.0.

Studies with the polyamines have shown dramatic increases in enzyme activity for assays run at low ionic strength while only modest enhancement of activity was observed at high ionic strength. The specificity of the nuclease was unaffected by the polyamines with either synthetic nucleotides or RNA as substrate. Inhibition of the enzyme by the ordered polynucleotides was reversed using spermidine or spermine, while other cations tested had no effect. The C ribonuclease was shown to bind to the polyamines and to polyguanylic acid. Tryptic digestion of the poly G-bound enzyme gave peptides bound to poly G which were released using excess spermidine. Three spermidine-bound peptides were isolated after ion exchange chromatography and the nucleophilic amino acids, serine, glutamic acid and aspartic acid were shown to be present in each peptide after amino acid analysis.

The polyamines were also shown to activate *Staphylococcal* nuclease for degradation of both RNA and DNA in the absence of calcium ion. If low concentrations of calcium ion are added to the polyamine-activated enzyme, up to double the expected activity (based on additive values) is observed. The polyamines also reversed inhibition of Staphylococcal nuclease by the ordered polynucleotides.

It is known that the polyamines are present in high concentrations in the urine of cancer patients. It is of great interest to determine how the polyamines affect the nucleases and their role in controlling the concentration of RNA in the cell. This information would be useful in controlling the growth rate of rapidly proliferating cells.

The projected course is to determine more fully the nature of the binding of spermiding to the isolated peptides and to examine the universality of these peptides as binding sites for large ordered polynucleotide complexes.

Keyword Descriptors

Enterobacter ribonuclease, polynucleotide inhibition, polyamines, Staphylococcal nuclease.

Publications

Levy, C. C., and Goldman, P. Residue specificity of a ribonuclease which hydrolyzes polycytidylic acid. J. Biol. Chem. 245: 3257-3262, 1970.

Levy, C. C. Enterobacter ribonuclease. II. A reexamination of the enzyme's specificity toward internucleotide linkages. Biochim. Biophys. Acta 246: 476-486, 1971.

Marotta, C. A., Levy, C. C., Weissman, S. M. and Varricchio, F. Preferred sites of digestion of a ribonuclease from Enterobacter sp. in the sequence analysis of Bacillus stearothermophilus 5S ribonucleic acid. Biochemistry 12: 2901-2904, 1973.

Frank, J. J., Hawk, I. A., and Levy, C. C. Polyamine activation of Staphylococcal nuclease. Biochim. Biophys. Acta (in press) 1975.

Project No. ZO1 CM 06933-01 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Influence of Renal Insufficiency on Levels of Serum Ribonuclease in Patients with Multiple Myeloma

Principal Investigator: T. P. Karpetsky

Other Investigators : C. C. Levy, R. L. Humphrey

Cooperating Units : Baltimore City Hospitals, Oncology Department

Man Years (July 1, 1974, through June 30, 1975)

Total : 0.3
Professional : 0.3
Other : 0

Project Description

The purpose of this study was to test the correlation between serum ribonuclease (RNase) levels and status of cancer in patients with multiple myeloma, as determined from complete patient medical records. RNase was measured in 34 patients with far advanced multiple myeloma. All had bone marrow plasmacytosis >50% of nucleated cells and all studies were done at least four weeks after previous chemotherapy. RNase levels ranged from 12 to 456 units/ml/min (1 u = A_{260} increment of 1.0) and the enzyme was specific for poly C. All of 15 patients with creatinine clearance (C_{CR}) <50 ml/min had an elevated RNase level (>45 u/ml/min had an elevated RNase level (>45 u/ml/min). Twelve of 19 patients with C_{CR} >50 ml/min had normal RNase. The RNase ranged from 50-117 u/ml/min in the remaining 7 patients with C_{CR} >50. A strong inverse correlation was therefore observed between the degree of renal insufficiency (C_{CR} values) and serum RNase levels. Several myeloma patients were studied throughout the course of their illness and the rise and fall of RNase was clearly related to the status of their renal function. Normal controls and patients with severe renal insufficiency but without plasma cell tumors were also studied. The overall finding of this study is that RNase levels are not a biomarker for the extent of plasma cell tumor, but are an indicator of renal function. It is proposed to continue this study by examining the relationship of polyamine levels to levels of RNase in patients with multiple myeloma and severe renal disease and thus establish whether or not elevated polyamine levels are a biomarker for multiple myeloma.

Keyword Descriptors

Ribonuclease, multiple myeloma, renal insufficiency, creatinine clearance, biomarker.

Publications - None

Project No. Z01 CM 06934-01 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Poly A Inhibition of Ribonuclease Associated with an Endogenous Inhibitor: Effects of Spermidine and Ionic Strength

Principal Investigator: T. P. Karpetsky

Other Investigators : C. C. Levy, P. A. Hieter

Man Years (July 1, 1974, through June 30, 1975)

Total	0.3
Professional	0.3
Other	0

Project Description

In order to establish the mechanism of inhibition by poly A of ribonucleases (RNases) from human sources, a model system isolated from Citrobacter sp. was studied. This bacterial RNase is complexed to an endogenous inhibitor which acts in a competitive manner with respect to the substrate, poly U. The effect of the inhibitor on enzymatic activity is strongly influenced by ionic strength. Increasing concentrations of Tris-HCl buffer (pH 7.1) reverses inhibition by increasing K_i alone. Although potassium phosphate buffer (pH 7.1) will also reverse inhibition, both K_i and Vm are affected by gradients of this salt. Kinetic mechanisms were established for the effects of both buffers. Spermidine will reverse inhibition of the RNase, but at much lower concentrations than required for an effect based solely on ionic strength. The plots of v vs spermidine are sigmoidal and the reciprocal plots are non-linear. Thus the kinetic evidence suggests a mechanism in which more than one spermidine binds to the enzyme-inhibitor complex, giving rise to a number od enzyme-spermidine forms that can catalyze the substrate-product conversion. The association of the enzyme with inhibitor lengthens the half-life of denaturation at 37° and therefore protects the enzyme from thermal inactivation. This endogenous inhibitor is therefore of prime importance in regulating the amount of enzymatic activity available at a given time. Furthermore, poly A was also shown to be a competitive inhibitor of this enzyme. The effects of poly A are reversed by raising the ionic strength or increasing the concentration of spermidine. Although the gross effects and responses to ionic strength and spermidine of poly A and the endogenous inhibitor are similar, these two substances were shown to be distinct. Work is currently in progress to establish the identity of the endogenous inhibitor. Attention is now focused on an enzyme from human sources specific for poly C. The effects of poly A, ionic strength and the polyamines on enzymatic activity are being pursued and computer-based kinetic models are being established for the interaction of this substrate-inhibited enzyme with these substances.

Keyword Descriptors

Poly A, polyamines, ribonuclease, regulation, competitive inhibition, ionic strength effects, mechanisms

Publications - none

Project No. ZO1 CM 06935-01 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : Isolation and Characterization of a Human Liver Ribonuclease

Principal Investigator: J. J. Frank

Other Investigators : C. C. Levy

Man Years (July 1, 1974, through June 30, 1975)

Total : 1/2
Professional : 1/2
Other : 0

Project Description

The purpose was to isolate and characterize a human liver ribonuclease to use for an investigation of factors contributing to control of RNA levels in the cell.

A ribonuclease has been purified 1500-fold from the 100,000 g supernatant of homogenized human liver cells. Standard purification procedures were used with a poly G affinity column as the final step. The pH maxima in phosphate buffer is a pH 6.4. Extensive hydrolysis of poly U produces 2',3' cyclic phosphates. Prolonged digestion of C RNA indicated that 80% of the products consisted of oligonucleotides of less than 10 nucleotides in length. The 5' termini of the oligonucleotides were found to be the nucleosides, adenosine and guanosine, in a ratio of 2:3.

Studies with the polyamines have indicated that enzyme activity can be increased dramatically in assays run at low ionic strength. The polyamines can also reverse inhibition of enzyme activity by the polynucleotides at low ionic strength.

Since ribonuclease activity is a major factor in the determination of RNA levels in the cell, it is of interest to determine those factors which can affect RNase activity. With this information control of the turnover of RNA in rapidly proliferating cells might be possible.

The characterization of the human liver ribonuclease will be completed. Further investigation of the control of enzyme activity by synthetic polynucleotides and cellular counterparts will be undertaken.

Keyword Descriptors

Liver ribonuclease, polyamines, enzyme purification, polynucleotide inhibitors.

Publications -

None.

Project No. Z01 CM 06936-01 BCRC
1. Pharmacology
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974, through June 30, 1975

Project Title : The Properties of Protein Inhibitors of Ribonuclease Activity

Principal Investigator: J. J. Frank

Other Investigators : C. C. Levy

Man Years (July 1, 1974, through June 30, 1975)

Total : 1/2
Professional : 1/2
Other : 0

Project Description

The purpose of the project is to investigate the mechanism of protein inhibition of ribonuclease activity, the stability of protein inhibitors and the control of inhibitor activity.

Protein inhibitors of nuclease activity have been isolated from rat liver and bovine lens. Kinetic studies indicate that the inhibition is of a non-competitive nature. The inhibitors were found to be unstable to freezing and thawing but could be stabilized with albumin. Preliminary studies with putrescine, spermidine and spermine indicate that protein inhibition of nuclease activity is enhanced by these agents.

The inhibitors are of interest as regulating agents of nuclease activity and therefore RNA levels in cells. An understanding of their mechanism of action would be useful in preventing the rapid proliferation of cancer cells.

The projected course is to look at the kinetics of denaturation of the protein inhibitors and to pursue the investigation of the polyamines and their effect on inhibitor activity.

Keyword Descriptors

Protein inhibitor, ribonuclease, polyamines.

Publications

None.

Project No. Z01 CM 06020-09 BCRC
1. Laboratory of Pharmacology
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Mechanism of Human Lymphocyte Reactivity to Non-Self

Previous Serial Number: NCI-6020

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: John C. Ruckdeschel, M.D.
Richard A. Winchurch, Ph.D.
J. Leonard Lichtenfeld, M.D.

Cooperating Units: None

Man Years:

Total:	2
Professional:	1 1/2
Others:	1/2

Project Description:

Continued work has been carried out to utilize immunologically induced in vitro lymphocyte responsiveness as a model of human lymphocyte reactivity to non-self. Several important advances have been made which relate both generally to evaluation of host immune responsiveness, the viral infectious disease area, and more specifically, to applications of this assay for evaluation of lymphocyte reactivity to putative oncogenic viruses. Previous work outside this laboratory has indicated that measles (rubeola) caused suppression of lymphocyte responsiveness in the blastogenesis system. More importantly, a routine assay for lymphocyte responsivness to measles did not exist because of these findings. This laboratory has presented evidence that purified (banded) virus is not capable of eliciting responsiveness in this system but responsiveness can be observed when the cell membrane preparation from which the virus was purified is used as the antigen in this system. This finding is of extreme importance because it points to the need for membrane associated antigen as the recognition template (either viral or cell) in order for lymphocyte responsiveness to be achieved. Application of this observation to other viruses has indicated that a viral infected cell membrane preparation is generally the best antigenic source to determine previous host exposure to virus in the lymphocyte blastogenesis system. Since lymphocyte responsiveness is dependent upon memory and not upon constant exposure of the host to antigen (that generally needed for antibody) the current accomplishments will allow for a broader investigation in the determination of previous exposure to putative oncogenic viruses.

In order to improve the functional applicability of the lymphocyte blastogenesis system we have applied the use of laser cytometry to evaluate cell change in this system. Laser cytometry has not only allowed for greater sensitivity in evaluating lymphocyte responsiveness in this system but has also added significant information concerning the nature of this response. Generally, it has been accepted that the lymphoblasts were the main result of exposure of a lymphocyte population to antigen, mitogen or allogeneic cells. Use of laser cytometry has revealed that the number of blast cells occurring secondary to response to antigen and mitogen were essentially similar and indicated further that a great number of small lymphocytes were also produced. The sensitivity of laser cytometric analysis appears somewhat better than that of tritiated thymidine and affords a direct and rapid non-isotope technique for the evaluation of cellular responsiveness. It is a major advance in technology within this field. Concurrent work demonstrated the effectiveness of levamisole to modulate lymphocyte responsiveness in the blastogenesis system. In addition, a correlation of PHA induced polyamine change in lymphocytes in relation to tritiated thymidine incorporation has also been carried out.

Keyword Descriptors: Lymphocytes; blastogenesis; laser; rubeola; cytometry; virus; polyamines; levamisole; membrane; PHA; lymphoblasts.

Honors and Awards: None

Publications:

Graziano, K.D., Levy, C.C., Schmukler, M., and Mardiney, M.R., Jr.: Parameters for Effective Use of Synthetic Double Stranded Polynucleotides in the Amplification of Immunologically Induced Lymphocyte Tritiated Thymidine Incorporation. Cellular Immunology 11:47-56, 1974.

Graziano, K.D., Marton, L.J., Mardiney, M.R., Jr., and LeGendre, S.: The Correlation of PHA Induced Polyamine Changes in Human Lymphocytes with Tritiated Thymidine Incorporation. Biomedicine 21:197-201, 1974.

Graziano, K.D., Ruckdeschel, J.C., and Mardiney, M.R., Jr.: Cell Associated Immunity to Measles (Rubeola): The Demonstration of In Vitro Lymphocyte Tritiated Thymidine Incorporation in Response to Measles Complement Fixation Antigen. Cellular Immunology 15:347-359, 1975.

Ruckdeschel, J.C., Graziano, K.D., and Mardiney, M.R., Jr.: Additional Evidence that the Cell Associated Immune System is the Primary Host Defense Against Measles (Rubeola). Cellular Immunology, June, 1975.

Lichtenfeld, J.L., Desner, M.R., Wiernik, P.H., Moore, S., and Mardiney, M.R., Jr.: Amplification of Human Lymphocyte Responsiveness to Immunologic Stimuli in Vitro by Levamisole. Journal of the National Cancer Institute Monograph "Modulation of Host Immune Resistance in the Prevention and Treatment of Induced Neoplasias" Michael A. Chirigos, editor, in press, 1975.

Project No. Z01 CM 06249-03 BCRC
1. Laboratory of Pharmacology
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients

Previous Serial Number: NCI-6249

Principal Investigator: John C. Sutherland, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.

Cooperating Units: Department of Pathology, University of Maryland Hospital
Baltimore, Maryland

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

An attempt has been made to ascertain if the alternate pathway of complement fixation might be responsible for the deposition of the third component of complement in kidneys morphologically normal by light microscopy postmortem. A total of 21 kidneys were available in which complement with or without gammaglobulin could be demonstrated in glomeruli by immunofluorescence. These were stained with antisera monospecific for IgA, IgG, IgM, C1Q, C3, C4, and properdin. Seven kidneys were from patients with acute leukemia, 1 with chronic leukemia, 7 with lymphomas, 1 with multiple myeloma, 4 with solid tumors, and 1 with no evidence of neoplasia. These patients could be divided into roughly three groups on the basis of immunofluorescent staining. Seven had only C3 and no immunoglobulins or early active components (Group A). Seven had immunoglobulins but no early active components (Group B). Six had both immunoglobulins and early active components (Group C). One had no immunoglobulins but at least one early active component. All had C3. Properdin was identified in two kidneys in group A, four in Group B and three in Group C. The present work compared to other studies suggest that the alternate pathway may have been selectively operative in groups A and B and "recruited" in half of group C.

Keyword Descriptors: Immune complex; deposition; glomerular nephritis; complement; alternate pathway; leukemia; lymphoma; slow viral disease.

Honors and Awards: None

Publications:

Sutherland, J.C., Markham, R.V., Jr., Ramsey, H.E., and Mardiney, M.R., Jr : Subclinical Immune Complex Nephritis in Patients with Hodgkin's Disease. Cancer Research 34:1179-1181, 1974.

Sutherland, J.C., and Mardiney, M.R., Jr.: Subclinical Immune Complex in the Glomeruli of Postmortem Kidneys. American Journal of Medicine 57: 536-541, 1974.

Project No. Z01 CM 06274-05 BCRC
1. Laboratory of Pharmacology
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody: The Characteristics of Reactivity with the Complement System in Vivo and Subsequent Effects.

Previous Serial Number: NCI-6274

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake
Eugene F. Cimino

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Previous work in which antibody has been characterized as being tumor specific has used standard assays of absorption both in vitro and in vivo. These assays have essentially established tumor antibody specificity when normal cells are not capable of absorbing out such activity either in vivo or in vitro. In the course of our investigation as to the lack of efficacy of tumor specific antibody in effectively mediating significant tumor kill in vivo we have established certain important parameters in relation to the interaction of antibody and complement. Of most importance is the observation that tumor antibody established to be specific by standard absorption techniques can be made to cross-react to normal (non-tumor) antigenic determinants when an absorption is performed in the presence of an active homologous complement source. This has been demonstrated in vitro and now characterized to occur in vivo. This finding creates a double edged sword in relation to the effective use of antibody in vivo in a situation in which complement is necessary to mediate lysis. If such antibody is utilized in a system in which the host has active complement activity but in which the complement is not homologous to the antibody, then specific binding to tumor takes place but no lytic effect is seen. In contrast if an active complement system is on board which can mediate lysis of tumor cells in the presence of the antibody being utilized, the administration of such antibody to such a host may result in specific binding of this antibody to normal cellular determinants - this occurring at a greater rate than that to tumor determinants because of the differential of antigenic concentration.

These findings explain in part the lack of efficacy of tumor specific antibody in vivo and allow for careful re-evaluation of possible utilization of antibody in the cancer therapeutic situation.

Keyword Descriptors: Antibody; complement; cross reactivity; tumor antigen; immunotherapy; serotherapy; cancer.

Honors and Awards: None

Publications:

Drake, W.P., and Mardiney, M.R., Jr.: Parameters of Serum Complement in Relation to Tumor Therapy. Biomedicine 21:206-209, 1974.

Drake, W.P., Pokorney, D.R., and Mardiney, M.R., Jr.: In Vivo Abrogation of Serum C3 and C5 by Administration of Cobra Venom Factor and Heterologous Anti-C3. Journal of Immunological Methods 6:61-72, 1974.

Drake, W.P., and Mardiney, M.R., Jr.: Complement-Mediated Alteration of Antibody Specificity in Vivo. Journal of Immunology 114:1052-1057, 1975.

Project No. Z01 CM 06292-03 BCRC
1. Laboratory of Pharmacology
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Immune Adjuvants

Previous Serial Number: NCI-6292

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake
W. Jefferson Pendergrast, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Studies in this laboratory focusing on the defining the potential and mechanism of action of various immune adjuvants in the prevention and treatment of neoplasia has revealed several important findings. Previous work has demonstrated the effectiveness of the chronic administration of polyadenylic-polyuridylic acid in prevention of leukemia in the AKR mouse. In order to define whether the effect of poly A:U in this situation was dependent on the age of the mouse at the time of administration, a study was carried out in which several groups of randomized AKR mice were treated weekly - the first group starting at 8 weeks of age and varying the initiation of therapy in the other groups every 2 months until 8 months of age. In this setting, a clear relationship of increased survival to early onset of therapy was revealed.

Additional work has explored the generally accepted concept that immunotherapy is most effective when the tumor burden is at its lowest point. Results available through the study of 2 transplantable tumor models (the AKR leukemia transplanted into the AKR and the B16 melanoma transplanted into the C57BL/6) indicate that immunotherapy initiated at a point of low tumor burden results in decreased survival as compared to a situation in which immunotherapy is initiated when a greater tumor burden exists.

This work is of importance because it brings to the forefront the concept that tumor specific antigenic concentration must be sufficient for the host to respond successfully to such a tumor. This concept is not extraordinary when one considers the absence of strong non-self antigens on autochthonous tumor cells. Continued work in this area is further defining the overall scheme in which immunotherapy can be effectively employed in relation to chemotherapy as well as surgery.

Keyword Descriptors: Immunotherapy; immune adjuvant; AKR leukemia; Tumor burden; B16 melanoma; AKR mouse; antigen concentration; polyadenylic-polyuridylic acid.

Honors and Awards: None

Publications:

Drake, W.P., Cimino, E.F., Mardiney, M.R., Jr., and Sutherland, J.C.: Prophylactic Therapy of Spontaneous Leukemia in AKR Mice by Polyadenylic-Polyuridylic Acid (poly A;U). Journal of the National Cancer Institute 941-944, 1974.

Desner, M.R., Lichtenfeld, J.L., Dennison, C.L., Kalmbach, K.O., and Mardiney, M.R., Jr.: A New Apparatus for BCG Scarification. Journal of the National Cancer Institute 54:57-60, 1975.

Project No. Z01 CM 06932-01 BCRC
1. Laboratory of Pharmacology
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: The Correlation of Abnormal Nuclease Activity to the Prediction and Presence of Cancer

Previous Serial Number: None

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators:
Walter P. Drake
W. Jefferson Pendergrast, Jr., M.D.
J. Leonard Lichtenfeld, M.D.
Carl C. Levy, Ph.D.
Morton Schmukler, M.D.

Cooperating Units: Section of Enzymology and Drug Metabolism

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Studies in immune adjuvants (NCI-6292) have suggested that abnormal ribonuclease activity existed in mice with neoplasia. Pursuit of information in this area has caused us to utilize a broad range of synthetic substrates to measure ribonuclease activity in the lymphoid cells and sera of genetically cancer prone, normal, and tumor bearing mice, as well as guinea pigs bearing tumor, and patients with neoplasia. The results have indicated: 1) A disparity exists in the ribonuclease activity in cell-free thymus homogenates in 5 strains of mice genetically predisposed towards leukemia or reticulum cell neoplasms (AKR, C58, PL, RF, and SJL) as compared to low tumor incidence animals (NZB, C57BL/6, Balb/c and DBA/2). Such activity was directed against polyuridylic acid and was observed in 8 week old mice, well before the onset of neoplastic transformation. Similarly, white blood cell ribonuclease activity was elevated in mice of the strains AKR, C3H/He, PL and RF). 2) RNA activity was studied in 6 transplantable murine tumor models. Nuclease activity directed against the synthetic substrates, polyadenylic acid, polyuridylic acid, and polycytidylic acid was measured and the data obtained for tumor-bearing animals was compared to their normal counterpart. Elevated activity against polyuridylic acid was observed in the plasma of all tumor bearing mice. RNase levels in both the spleen and thymus were generally altered as well. 3) When the ribonuclease activity observed in the sera of L2C leukemic guinea pigs was compared to that activity observed in white blood cell lysates of identical animals, white blood cell associated ribonuclease activity directed against polyuridylic acid decreased with the progression of neoplastic disease although serum ribonuclease activity remained unchanged. 4) When the serum of 100 patients with a variety of malignancies (acute non-lymphocytic leukemia, brain tumor, Hodgkin's Disease, and non-Hodgkin's lymphoma) were evaluated for ribonuclease activity against 6 synthetic polynucleotides approximately 70% of the patients exhibited elevated serum ribonuclease activity at the 95% confidence level when compared to 30 controls.

This accumulation of findings i.e. the elevation of nuclease activity in the thymus of high incidence tumor strain mice, the elevation of nuclease activity in the serum and white cells of mice with transplantable leukemia, the depression of nuclease activity in the white cells of guinea pigs bearing the L2C leukemia, and the elevation of serum nuclease activity in patients with neoplasia indicate that alteration in nucleolytic activity represents an important biochemical marker for the potential development of neoplasia as well as the presence of neoplastic disease.

Keyword Descriptors: Ribonuclease; polyuridylic acid; polyadenylic acid; polycytidylic acid; leukemia, Hodgkin's disease; acute non-lymphocytic leukemia; non-Hodgkin's lymphoma; brain tumor; L2C leukemia; AKR.

Honors and Awards: None

Publications:

Drake, W.P., Kopyta, L.P., Levy, C.C., and Mardiney, M.R., Jr.: Alterations in Ribonuclease Activities in the Plasma, Spleen and Thymus of Tumor-Bearing Mice. Cancer Research 35:322-324, 1975.

Drake, W.P., Pokorney, D.R., Chipman, S., Levy, C.C., and Mardiney, M.R., Jr.: Elevated Ribonuclease Activity in the Thymus and White Blood Cells of Genetically Cancer Prone Mice. Journal of Experimental Medicine, April, 1975.

Drake, W.P., Pokorney, D.R., Ruckdeschel, J.C., and Mardiney, M.R., Jr.: A White Blood Cell Ribonuclease Assay for the Possible Monitoring of Malignancy. Journal of the National Cancer Institute, April, 1975.

Project No.: Z01 CM 06250-02 BCRC
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural and Tissue Culture Studies of Leukemic Cells of Patients before and during treatment and of Normal Donor granulocytes harvested for transfusion therapy.

Previous Serial Number: NCI-6250

Principal Investigator: Frances T. Sanel

Other Investigators: Peter H. Wiernik
Joseph Aisner
Charles A. Schiffer
Sheldon Glusman

Cooperating Units: Tumor Service, BCRC, NCI
Pathology Dept., Good Samaritan Hospital

Man Years:	Total:	1/4
	Professional:	1/8
	Other:	5/8

Project Description:

To evaluate the suitability for transfusion of normal granulocytes harvested on nylon fibres and treated by various techniques in a model system to restore them to levels comparable to control cells in micromorphology, viability and function, and to, if possible, eliminate factors inducing adverse reactions in recipients. Cells obtained by filtration leucapheresis using current methods were demonstrated by SEM, TEM, cytochemical and functional testing to be altered during harvesting and processing for transfusion. In a model system, we demonstrated that washing harvested cells in HBSS and brief incubation in heparinized autologous plasma significantly restored them structurally and functionally. Preliminary clinical trials of this method have been made and results are encouraging.

Continuing studies are directed toward the characterization of leukemias of questioned identity by ultrastructure, cytochemistry and function of peripheral blood and bone marrow cells. Hairy cell leukemia should be definitively identified in suspected cases since the prognosis for untreated patients with this disease is considerably better than for those treated.

In a case submitted for our study, we demonstrated the accepted micro-morphological criteria for Hairy cells, mononuclear clear cells with numerous cytoplasmic projections at the periphery. Zipper-like junctions were demonstrated in these cells as well as their ability to phagocytose microorganisms. These are properties of cells of the monocytic series, and strongly suggest the non-lymphoid origin of Hairy cells.

We will continue our investigation of methods to restore the maximal viability and efficiency of granulocytes collected for transfusion therapy. Cytochemical study of the glycocalyx of leukemic cells and utilization of SEM and TEM to elucidate the zipper-like junctions in leukemic monocytes and Hairy cell leukemias will be pursued. Tissue culture of precursor cells to induce differentiation and responsiveness of these cells to chemotherapeutic agents is planned.

Key Descriptors: Non-lymphoid Leukemias, SEM, TEM and TC; Hairy cell Leukemia; Donor Granulocyte Repair; Granulocyte Transfusion

Presentations:

Sanel, F.T. and Glusman, S., Hairy Cell Leukemia: Ultrastructural and Functional Studies. Washington Society of Electron Microscopists, May 1975

Sanel, F.T., J. Aisner, C.J. Tillman, C.A. Schiffer, and P.H. Wiernik., Restoration of Granulocytes Harvested by Filtration Leucapheresis. International Symposium on Leucocyte Separation and Transfusion, London, September 1974

Publications:

Sanel, F.T., J. Aisner, C.A. Schiffer, and P.H. Wiernik., Evaluation of Granulocytes Harvested by Filtration Leucapheresis: Functional, Histochemical, and Ultrastructural Studies in: Leucocyte Separation and Transfusion (ed., John Goldman) Academic Press (London 1975)

Sanel, F.T., J. Aisner, C.A. Schiffer, and P.H. Wiernik., Structure and Function of Granulocytes Collected on Nylon Fibres and Harvested for Transfusion Therapy, J. Cell Biol. 63: 297a, 1974

Schiffer, C.A., F.T. Sanel, B.K. Stechmiller and P.H. Wiernik., Functional and Morphologic Characteristics of the Leukemic Cells of A Patient with Acute Monocytic Leukemia: Correlation with Clinical Features, Blood (in press) 1975

Project No.: Z01 CM 06272-04 BCRC
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Studies of Myelomonocytic Leukemia in an Animal Model System: Light and Electron Microscopy, Biological Experiments and Chemotherapy

Previous Serial Number: NCI-6272

Principal Investigator: Frances T. Sanel

Other Investigators: L. Lichtenfeld
P. H. Wiernik

Cooperating Units: Medicine Service, BCRC, NCI

Man Years (July 1, 1974 to June 30, 1975)

Total:	1/2
Professional:	1/8
Other:	3/8

Project Description:

To study etiology, passaging under varying conditions and trans-species grafting of transplantable, muramidase-producing myelomonocytic WEHI-3 virus producing cells utilizing techniques of short term tissue culture, hematological histological, histochemical and ultrastructural techniques; precise identification of the specific virus type within the murine oncorna virus group, and further evaluation of their oncogenic capacity; to determine the phagocytic competence of myelomonocytic neoplastic cells whose normal counterparts actively ingest particulates; to determine the effect of Levamisole (Ortho) on tumor induction and/or regression.

Scanning electron microscopy (SEM) of tissue cultured WEHI-3 tumor cells additionally confirmed their identity as monocyte-macrophage type cells and transmission (TEM) demonstrated their production of "C" type particles, previously tested in tumor extracts that demonstrated group specific antigens of murine leukemia NB tropic viruses of the FMR group, with high titers of infectivity. Newborn BALB/c mice injected with NB tropic cell-free tumor filtrates in many instances developed hind leg paralysis with concommittant lymphoma or with no other manifestation of disease. TEM of lumbar spinal cord samples revealed moderate demyelinization and extra-cellular C-type viruses. TEM of decalcified femurs of osteopetrotic animals revealed numerous C-type particles in lacunar areas where osteocytes were embedded in bone matrix. Transplacental transmission of viruses by injection of pregnant females with cell free filtrates (CFF) resulted in lymphomas in both mothers and offspring after latencies of 6 to 10 months. Injection of CFF into normal 10 month old BALB/c males induced lymphomas after a 6 to 8 month latency. In preliminary chemotherapeutic studies with Levamisole (Ortho), the drug was administered ip, in saline, 5 μg per mouse at staged intervals before tumor grafting, simultaneous with tumor grafting, and after tumor grafting, and/or in multiple doses of 5 μg each. Latency periods of tumor development did not significantly differ from tumor injected controls.

These studies have established that transplacental passage of WEHI-3 tumor viruses induce lymphomas in offspring suggesting that immunologic responses to them have not been elicited; that adult BALB/c mice cannot reject these tumor-inducing viruses, unlike adult AKR mice injected with Gross virus; that these viruses are in some instances sequestered and/or produced within the bone matrix, and that demyelinization of spinal cord axons and the presence of virus in the spinal cord may be related phenomena in animals that develop the hind leg paralysis. We will continue to study the oncogenic expression of these viruses and will investigate in vivo and in vitro chemitherapeutic anti-viral and anti-neoplastic agents.

Key Descriptors:	animal model of AMML, oncogenic virus, transplacental transmission of oncogenic NB tropic virus, CFF induction of lymphoma in adult BALB/c mice; SEM, TEM and TC of WEHI-3 cells, Chemotherapy
Presentations:	Studies of Myelomonocytic Leukemia in BALB/c Mice - Grand Rounds, BCRC, NCI, January 1975 SEM and TEM of Decalcified Osteopetrotic Femurs in BALB/c mice - Washington Society of Electron Microscopy, May 1975

Project No.: Z01 CM 06923-01 BCRC
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Fine Structure and Cytochemistry of Human Neoplastic Solid Tumors

Previous Serial Number: NCI 6923

Principal Investigator: Frances T. Sanel

Other Investigators: Arthur Schantz

Cooperating Units: Department of Pathology

Man Years: Total: 1/8
Professional: 1/8
Other: 3/4

Project Description:

To study with the collaboration of consulting pathologists, surgical specimens of neoplastic cells and tissues that are difficult to diagnose by conventional histochemical methods and/or those not previously described in the literature. In the present study of a surgical specimen of a solid tumor, a diagnosis of undifferentiated nasopharyngeal carcinoma of entodermal origin was made on the basis of ultrastructural and cytochemical studies differentiating the tumor cells from those of possible chordoma. Such studies that describe, characterize and identify aberrant neoplastic cells are an aid to prognosis and treatment.

Key Descriptors: Tumor of entodermal origin, E.M., Histochemistry

Project No.: Z01 CM 06924-01 BCRC
1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1974 through June 30, 1975

Project Title: Ultrastructural Studies of Aberrant Cells in Possible Preleukemic States

Previous Serial Number: NCI 6924

Principal Investigator: Frances T. Sanel
J. Breton-Gorius

Other Investigators: G. Flandrin, M. Y. Daniel, J. Chevalier, M. Lebeau

Cooperating Units: Institute de Recherches Sur Les Maladies du Sang, Creteil and Paris, France

Man Years (July 1, 1974 to June 30, 1975)
Total: 1/8
Professional: 1/8
Other: 3/4

Project Description:

To study ahd characterize the abnormal erythroid precursors from the bone marrow of a patient with refractory anemia and ineffective erythropoiesis. Ultrastructural studies in our laboratories of material sent to us from France, revealed unusual junctional complexes between aberrant mono-and multinucleated erythroid precursors and similar seemingly intracellular structures resembling the zipper-like junctions we reported (Science 168, 1970) in human leukemic monocytes.

Continuing ultrastructural and cytochemical studies are in progress on blood cells and bone marrow cells from patients exhibiting possible hematologic dyscrasias in order to elicit, if possible predictive factors toward definitive diagnosis that would aid in prognosis and treatment.

Key Descriptors: Zipper-like junctions; Refractory Anemia; Preleukemic States

ANNUAL REPORT

OFFICE OF THE ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Program is responsible for coordinating and monitoring the clinical contracts program of the DCT as well as coordinating these activities with similar activities in other NCI programs. This is accomplished by the Investigational Drug Branch (Milan Slavik, M. D., Ph. D., Chief) and the Combined Modality Branch (Stephen K. Carter, M. D., Chief).

Clinical Contracts Program

The Clinical Contracts Program of the Division of Cancer Treatment has been developed to implement the two major clinical thrusts of the division:

(1) Clinical evaluation of new anticancer drugs.

(2) Development of optimal therapy for specific tumor types by using combined modality approaches.

It is recognized that the final common pathway of any modality-oriented development is its application in a disease-oriented setting. Therefore, most of the DCT contracts are disease-oriented. This approach, by necessity, has modified the three classical phases of clinical drug testing since only Phase I trials can be considered completely modality-oriented and Phase II is the critical interacting point between the modality- and disease-oriented thrusts (Figure 1).

This increased emphasis on the integration of chemotherapy into a series of disease-oriented strategies leads to a conflict between the clinical resources needed by the Drug Development Program and the exigencies of devising an optimal combined modality treatment strategy for solid tumors. This conflict is most critical in allocating clinical resources in the advanced disease states of different tumors. For each tumor, Phase II resources may be used for investigating single agents, combination chemotherapy regimens, or even combined modalities (e. g., drugs plus immunotherapy or drugs plus radiotherapy). Single agents include established drugs that have not been tested against a given tumor, new schedules of established drugs, or investigational drugs. Investigational drugs may be those with some measure of established activity (e. g., nitrosoureas, adriamycin) or new drugs for which Phase I study has recently been completed.

Each tumor must be analyzed from the standpoint of what is required to develop an overall strategy for specific treatment. The drugs or regimens that are chosen for Phase II and III study in patients with advanced disease must integrate into this strategy. However, in many cases the strategy may directly conflict with the needs of a drug development program which places as many as 10 new drugs each year into clinical trial.

Currently, there are approximately 25 commercially available standard antitumor agents. The Drug Liaison and Distribution Section holds INDs for more than 50 anticancer drugs, many of which show some evidence of anticancer activity. The program currently has 10 drugs in Phase I study and, therefore, approximately 40 drugs that have passed Decision Network (DN) 2 in the linear array and are progressing toward eventual clinical trial. Since the input to the screen has been increasing, even the use of all the currently available clinical resources would be inadequate to meet the needs of the drug development linear array. Obviously, all the clinical resources cannot be used in this way and it is essential that an overall strategy be designed for using these precious resources. The DCT must make decisions in two critical areas:

(1) How many of the major solid tumor types will be included in the program and what priority should they have among themselves and in relation to ongoing programs in leukemia and lymphoma?

(2) How should the DCT allocate advanced disease resources, within the major solid tumors, between Phase II studies of new investigational drugs and other single agents or combination regimens? As a result of this kind of analysis, a decision must be reached on the number of new drugs arising from the drug development pool that can be handled. Lastly, and most difficult, is the question of how many drugs are actually best within the DCT overall treatment plan.

The clinical contract program can be outlined as follows:

(1) Phase I contracts designed to perform initial clinical evaluation of new drugs, and wherever possible, clinical pharmacology.

(2) Phase II contracts for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.

(3) Phase III contracts for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.

> In both Phase II and III the choice of regimens is determined by what is most needed to fit into the combined modality strategy for that tumor. In most contracts, Phase II and III are inseparable and are placed together in this paper for descriptive purposes

(4) Phase IV contracts for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.

A. Phase I Studies

A Phase I Working Group (Table 1) was formed about two years ago for the specific purpose of undertaking, in a coordinated fashion, all initial clinical evaluations of drugs sponsored for clinical trial by the DCT. The group has 11 member institutions, 7 of which are funded to a total of $850,000.

The group meets four times each year to present results of their current studies and to obtain information about new drugs that are available for study. An attempt has been made to have this group interact with the critical individuals working in preclinical drug development so that the latter can understand the clinical needs and problems and modify their studies accordingly where possible.

B. Disease-Oriented Studies

1. Lung Cancer. Lung cancer studies are supported by a series of contracts with individual institutions, either as part of an overall group (Lung Cancer Working Party, Table 2) or as independent investigators. In addition, a significant part of the Veterans Administration Cooperative Group work under the transfer of funds mechanism is involved in lung cancer studies. The contracts are outlined in Table 3.

2. Large Bowel (Table 4). Large bowel cancer is the area in which the first clinical contract was undertaken by the DCT nearly 5 years ago with the Mayo Clinic and Dr. Charles Moertel. This contract produced Phase II evaluations of a large number of single agents but, with the preliminary success of the methyl CCNU + 5-FU + vincristine combination and the tantalizing observations with MER, work has moved more into the Phase III area. It is hoped that the Phase II large bowel studies will be picked up by other contractors, especially the large solid tumor contractors. Combined modality studies will now become a major effort through a new RFP that will be integrated into the Gastrointestinal Tumor Study Group.

3. Breast Cancer (Table 5). The contract interest of the DCT in breast cancer is second in age only to large bowel cancer. Over the last few years these contracts have been fully integrated with the treatment contracts of the Breast Cancer Task Force since the project officers have been nearly identical in both cases. For this reason, the DCT work in this area appears relatively smaller when compared to the other major tumor types.

4. Pancreas. Pancreatic cancer was the first tumor type approached by the Gastrointestinal Tumor Study Group (Table 6) which was formed about 2-1/2 years ago to develop combined modality trials for the entire range of gastrointestinal cancer. The organizational concept for this group was to have a small highly selected group of institutions, each of which would send a member for each modality to plan trials for every stage of each disease type. The group studies in pancreatic cancer include 7 funded contracts at a total cost of approximately $800,000 (Table 7).

5. Stomach Cancer. Stomach cancer, still a major killer in the United States despite its declining incidence, has been relatively neglected in the U.S. clinical trials area. The major thrust in the DCT is through the Gastrointestinal Tumor Study Group in three on-going protocols, one for each stage of disease, with 6 contracts funded at a total yearly cost of $660,000 (Table 8).

6. Ovarian Cancer (Table 9). Until recently, contract-supported work in ovarian cancer was relegated to the Phase II-III area with 2 contracts totalling $220, 000. Because of the excellent potential for a successful combined modality attack on this disease, an ovarian cancer study group will be established from a selected group of institutions with extensive experience and combined modality expertise to design trials which could not be done in a single institution. Approximately $1, 000, 000 has been allocated to support this effort.

7. Brain Tumors. Brain tumor studies are performed totally by the Brain Tumor Study Group (Table 10) which was the first contract supported group established by the former Chemotherapy Program.

8. Testicular Cancer. Testicular tumor trials are supported by a Testicular Tumor Study Group formed to conduct combined modality studies for stage II disease (Table 11).

9. Malignant Melanoma. Studies in advanced malignant melanoma (Table 12) will be supported through the large solid tumor contracts and those in early disease will be conducted by the WHO Melanoma Study Group.

10. Other Solid Tumors. The remainder of the solid tumors will be studied through the large Phase II-III disseminated solid tumor contracts, of which three have been approved to date (Table 13). A search for more is planned.

As can be seen in Table 14 the total clinical contract program will total nearly 10 million dollars in FY 76 when all the planned resources have been funded.

Disease and Drug Oriented Analysis of Tumor Drug Interactions

Since the DCT is putting a high priority on the integration of chemotherapy into a combined modality program with surgery and/or radiotherapy, the CTEP has undertaken a detailed disease and drug oriented analysis of tumor-drug interactions of the major adult solid tumors. This analysis has aimed at elucidating the following:

(1) What drugs have and have not been tested.

(2) Which drugs are active and to what degree.

(3) What combinations have been shown to be superior to single agents.

(4) What combined modality approaches are now ongoing and with what components.

Table 15 is a broad outline cross-reference chart showing the status of clinical evaluation for 30 drugs against 17 solid tumors based on published and unpublished results on file in the CTEP. Each of the 30 drugs has shown definite evidence of activity against at least one tumor type from among the hematologic malignancies or solid tumors. Investigational drugs, which have not been adequately evaluated or have shown no evidence of activity in any tumor, and hormonal agents are excluded from this review.

The question of whether the evaluation of a particular drug in a specific tumor has been sufficient to allow a decision on its clinical activity is obviously an arbitrary one. Nevertheless, the drug-tumor interactions in Table 15 have been characterized by the staff of the CTEP through analysis of what is felt to be the maximum data available to any single group experienced in evaluating anticancer agents. The designation "NE" denotes drugs that have not been evaluated or situations where a drug has been inadequately evaluated and no decision can be made regarding its activity. A "-" indicates that adequate evaluation has taken place and the drug is inactive. On the other hand, some drugs have been evaluated to an extent permitting a "+" for evidence of activity, although it is not fully established, or "++" in cases where clinical activity is definitely established.

Table 16 contains the data used in making the clinical evaluations in Table 15. The figures provide the number of responders divided by the number of evaluable patients, and this fraction multiplied by 100 yields the percent response.

The best available definition of an evaluable patients is one who received the drug by an appropriate dose schedule. Similarly, the most reliable definition of response is 50% or greater shrinkage of a measurable lesion. Both of these definitions were most difficult to apply to data for older, now commercially available, agents. With time, the newer agents have undergone better defined trials with more carefully defined criteria of response in more disease-oriented trials. However, as the science, art, and practice of chemotherapy have advanced, the new drug trials have been in more advanced disease patients who have failed to respond to more vigorous chemotherapy, including combinations. Thus, we have tended to put more weight on the Phase II trials of the newer drugs with regard to efficacy data (a Phase II trial attempts to establish evidence of drug activity in a single tumor or many tumor types). There are few Phase III trials of the newer drugs as single agents in most tumor types. Instead, most of these involve combination chemotherapy studies with these drugs (a Phase III trial is designed to compare a new form of therapy with a standard form of therapy).

The data presented and our evaluations may be open to many questions and criticisms. However, it is the most defined data available and does permit some comparisons. We have specifically elected not to draw any conclusions here, as these have been and will continue to be presented elsewhere.

Table 17 presents a disease oriented analysis of the 17 tumors for which a decision on the activity of the 30 drugs is possible on the basis of adequate clinical evaluation. It shows that such a decision on more than half the drugs can be made for only 5 tumors. These include the three most common ones (colon, lung, and breast), head and neck cancer, and melanoma, an uncommon tumor that has been extensively studied because its lesions are readily accessible to measurement and analysis. Among the other 12 tumors, the percentage of drugs inadequately evaluated or completely untested ranges from 63% (sarcoma) to 100% (uterus).

Table 18 ranks the solid tumors by percentage of drugs having definite activity. Table 19 is a similar ranking but is based on drugs with evidence of activity as well as those with definite activity.

From a drug-oriented standpoint, Table 20 ranks the drugs with respect to the number of tumors in which each has been adequately evaluated. Only 8 drugs have received adequate testing in about half of the tumors. Inadequate testing, or none at all, is quite evident for a large number of agents. Table 21 ranks the drugs by the numbers of tumors where definite responsiveness has been observed; similarly, Table 22 ranks them by definite activity or evidence of activity.

The disease oriented review of single agent data, active combinations, and current combined modality trials in APPENDIX A shows that 48 combined modality protocols are now in progress in 11 solid tumor types. The combined modality trials are broken down by disease in Table 23.

Twenty-five (25) different chemotherapy regimens are being evaluated, as are 9 immunotherapy regimens and 7 regimens combining chemotherapy and immunotherapy. Also, there are 15 regimens employing chemotherapy plus radiotherapy.

Although this listing is not intended to be encyclopedic, it does show the wide range of studies being evaluated in attempts to improve survival by combined modality approaches in solid tumors. As the data accumulate and are analyzed we will begin to learn where successes and failures have occurred so we can plan more intelligently for future investigations.

HONORS AND AWARDS

1. Carter, S. K.: Civil Service Superior Service Award
2. Carter, S. K.: Appointed Chemotherapy Coordinator U. S. -Japan Agreement on cancer research.

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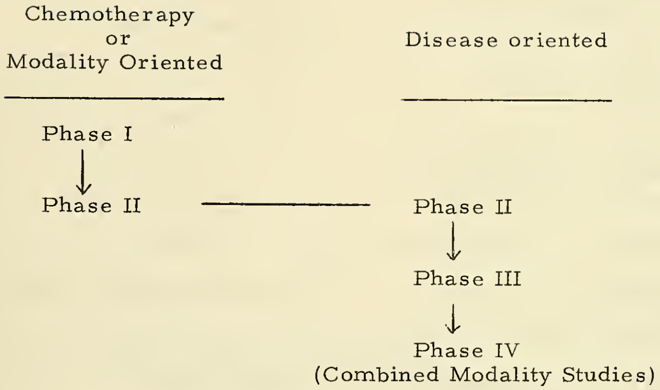

Figure 1. Phases of clinical drug testing in the DCT contract program.

Table 1. Phase I Working Group (Stephen K. Carter, Chairman).*

Institution	Principal Investigators
M. D. Anderson Hospital & Tumor Institute	Emil Freireich
Sidney Farber Cancer Center	Emil Frei III
Mayo Clinic	Charles Moertel
Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff
Mt. Sinai Hospital, New York	James Holland
Children's Hospital of Los Angeles	Myron Karon (deceased)
University of Kansas Medical Center	Barth Hoogstraten
Yale University School of Medicine†	Joseph Bertino
Roswell Park Memorial Institute †	Edward Henderson and Arnold Mittelman‡
St. Jude Children's Hospital†	Alvin Mauer
Emory University School of Medicine†	Charles Vogel

*Total funding - approx. $850,000
† Not funded
‡ Separate memberships

Table 2. Lung Cancer Working Party (Oleg Selawry, Chairman; Ronald Vincent, Executive Officer; Marvin Zelen, Statistician)*

Institution	Principal Investigators
Mayo Clinic [†]	David Carr & Robert Eagan
Roswell Park Memorial Institute	Ronald Vincent & Arnold Mittelman
M. D. Anderson Hospital & Tumor Institute	Clifton Mountain & Emil Freireich
Ohio State University Hospital	Karl Klassen & Henry Wilson
University of Capetown	Rossall Sealy
University of Liverpool	Roger Ryall
V. A. Medical Oncology Branch (NCI)	

[*] Total funding approximately $1,000,000
[†] Resigned

Table 3. Lung cancer contracts.

Clinical Studies	Institution or Group	Principal Investigators
Phase II-III	Lung Cancer Working Party	
	Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff & Robert Golbey
	V. A. Lung Cancer Study Group	Julius Wolf
Phase IV	Lung Cancer Working Party	
	V. A. Lung Cancer Study Group	
	V. A. Surgical Adjuvant Study Group	

Table 4. Large bowel cancer contracts.

Clinical Studies	Institution or Group	Principal Investigators
Phase II-III	Mayo Clinic*	Charles Moertel
	NCI, Milan	Gianni Bonadonna
	Memorial Hospital for Cancer & Allied Diseases †	Irwin Krakoff
	M. D. Anderson Hospital & Tumor Institute†	Jeffrey Gottlieb
	University of Leeds	Edward Cooper
Phase IV	Gastrointestinal Tumor Study Group‡	
	Veterans Administration Surgical Adjuvant Study Group	George Higgins

*$100,000
† Part of large solid tumor contract
‡ Responses to RFP currently being evaluated; estimated cost $1,000,000

Table 5. Breast cancer contracts.

Clinical Studies	Institution or Group	Principal Investigators
Phase II-III*	Mayo Clinic	David Ahmann
	Roswell Park Memorial Institute	Thomas Dao
	Albany Medical Center & Hospital	John Horton
	M. D. Anderson Hospital & Tumor Institute	George Blumenschein
	NCI, Milan	Gianni Bonadonna
	Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff
Phase IV	NCI, Milan	Umberto Veranesi & Gianni Bonadonna

*Estimated total - $750,000

Table 6. Gastrointestinal Tumor Study Group (Philip Schein and Charles Moertel, Chairmen; Marvin Zelen, Statistician).

Institution	Principal Investigators
Mayo Clinic	Charles Moertel
Roswell Park Memorial Institute	Arnold Mittelman
Sidney Farber Cancer Center	Emil Frei
Yale University	Howard Spiro
University of Southern California	Thomas Hall
U. C. L. A. *	Frank Sparks
University of Miami School of Medicine	Martin Kalser
Mt. Sinai Hospital, New York†	Howard Bruckner & James Holland

*Funded only for pancreatic cancer

†Funded only for gastric cancer

Table 7. Pancreatic cancer contracts.

Clinical Studies	Institute or Group	Principal Investigators
Phase II-III	Gastrointestinal Tumor Study Group	
	Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff
Phase IV	Gastrointestinal Tumor Study Group	
	V. A. Surgical Adjuvant Study Group	

Table 8. Stomach cancer contracts.

Clinical studies	Institution or Group	Principal Investigators
Phase II-III	Gastrointestinal Tumor Study Group	
	Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff
	NCI, Milan	Gianni Bonadonna
	University of Leeds	Edward Cooper
Phase IV	Gastrointestinal Tumor Study Group	
	V. A. Surgical Adjuvant Study Group	

Table 9. Ovarian cancer contracts.

Clinical Studies	Institution or Group	Principal Investigators
Phase II-III	M. D. Anderson Hospital & Tumor Institute	Julian Smith
	Mt. Sinai Hospital, New York	Ezra Greenspan
	Mayo Clinic*	David Decker
	Memorial Hospital for Cancer & Allied Diseases*	Manuel Ochoa
Phase IV	Ovarian Cancer Study Group (proposed)	

*Part of large solid tumor contract

Table 10. Brain Tumor Study Group (Michael Walker, Chairman; Edmund Gehan, Statistician).*

Institution	Principal Investigators
N. Y. U. Medical Center	Joseph Ransohoff
Ohio State University Hospital	William Hunt
Bowman-Gray School of Medicine	Eben Alexander
University of Connecticut Health Center	Guy Owens
University of Kentucky Medical Center	Horace Norell
University of Indiana	John Mealy
University of California (SF) School of Medicine	Charles Wilson
Duke University	Steven Mahaley
Veterans Administration †	

*Estimated total funds - $777,000

† Transfer of funds

Table 11. Testicular Tumor Study Group (Robert Golbey, Chairman). *

Institution	Principal Investigator
Roswell Park Memorial Institute	Claude Merrin
Memorial Hospital for Cancer & Allied Diseases	Robert Golbey
Mayo Cinic	R. G. Hahn
Bethesda Naval Hospital	Elliot Perlin
Walter Reed General Hospital	Johannes Blom

* Approximate cost - $150, 000.

Table 12. Malignant melanoma contracts.

Clinical Studies	Institution or Group	Principal Investigators
Phase II-III	M. D. Anderson Hospital & Tumor Institute*	Jeffrey Gottlieb
	Mayo Clinic*	David Ahmann
	Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff
Phase IV	WHO Melanoma Study Group	Umberto Veronesi

*Part of large solid tumor contract

Table 13. Phase II disseminated solid tumor contract (400 patients).*

Institution	Principal investigators
M. D. Anderson Hospital & Tumor Institute	Emil Freireich and Jeffrey Gottlieb
Mayo Clinic	John Edmondson and Robert Eagan
Memorial Hospital for Cancer & Allied Diseases	Irwin Krakoff and Robert Golbey

*Additional tumor types: Bladder cancer, prostate cancer, renal cancer, head and neck, sarcomas, cervical cancer, and endometrial cancer.

Table 14. Total clinical contract program costs, including new projects.

Clinical Contracts	Number	Approximate Cost
Phase I Working Group	7	850,000
Lung Cancer Working Party	6	1,000,000
Colon Cancer		
Phase II Studies	2	150,000
Surgical Adjuvant (Planned)		1,000,000*
Gastrointestinal Tumor Study Group		
Pancreas	7	800,000
Stomach	6	660,000*
Breast Cancer Phase II-III	5	550,000
Ovarian Cancer		
Phase II	2	220,000
Surgical Adjuvant (Planned)		1,000,000*
Brain Tumor Study Group	8	770,000*
Testicular Tumor Study Group	3	150,000
Melanoma	1	50,000
Phase II-III Disseminated Solid Tumors	3	1,200,000*
Hepatoma and African Studies	2	195,000
VA Transfer of Funds for Lung Cancer Study Group and Surgical Adjuvant Group		1,300,000
Foreign Clinical Studies (NCI, Milan)		75,000
	Total	9,970,000

*Projected costs

Table 15. Cross reference chart of drug-tumor interactions.

DRUGS	TUMOR TYPES																	TUMOR ACTIVITY Spectrum of Drugs			
	Colon	Lung	Breast	Pancreas	Ovary	Prostate	Stomach	Cervix	Head & Neck	Bladder	Kidney	Esophagus	Brain	Testicle	Melanoma	Sarcoma	Uterus	NE	-	+	++
ALKYLATING AGENTS																					
Cyclophosphamide	+	++	++	NE	++	NE	NE	++	+	NE	NE	NE	NE	NE	-	++	NE	9	1	2	5
Nitrogen Mustard	-	++	+	NE	NE	NE	+	NE	-	NE	NE	NE	NE	NE	-	NE	NE	11	3	2	1
Chlorambucil	-	NE	+	NE	++	NE	+	+	+	NE	NE	NE	NE	NE	-	+	NE	9	2	5	1
Melphalan	-	NE	++	NE	++	NE	NE	+	NE	NE	NE	NE	NE	+	-	NE	NE	11	2	2	2
Busulfen	NE	-	NE	NE	NE	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	2	0	0
ANTI-METABOLITES																					
5-Fluorouracil	++	-	++	++	++	+	++	++	+	+	-	NE	NE	NE	-	NE	NE	5	3	3	6
Methotrexate	+	++	++	NE	NE	NE	NE	+	++	NE	NE	NE	+	NE	-	NE	NE	10	1	3	3
6-Mercaptopurine	-	-	-	NE	NE	NE	NE	NE	+	NE	NE	NE	NE	NE	-	NE	NE	12	4	1	0
6-Thioguanine	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	16	1	0	0
Ara-C	-	-	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	-	NE	NE	13	4	0	0
MITOTIC INHIBITORS																					
Vincristine	-	-	++	NE	-	NE	NE	+	NE	NE	NE	NE	+	NE	-	++	NE	9	4	2	2
Vinblastine	-	-	++	NE	NE	NE	NE	NE	+	NE	NE	NE	+	++	-	NE	NE	10	3	2	2
ANTITUMOR ANTIBIOTICS																					
Actinomycin D	-	NE	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	++	+	++	NE	12	2	1	2
Mithramycin	NE	-	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	-	++	-	-	NE	11	5	0	1
Daunomycin	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
Adriamycin	-	++	++	NE	++	NE	+	+	+	++	-	NE	NE	+	-	++	NE	5	3	4	5
Bleomycin	-	-	-	NE	NE	NE	NE	+	++	NE	NE	+	NE	++	-	NE	NE	9	4	2	2
Mitomycin C	++	+	++	+	NE	NE	++	NE	NE	NE	NE	NE	NE	NE	-	+	NE	10	1	3	3
RANDOM SYNTHETICS AND MISCELLANEOUS																					
BCNU	+	+	+	-	NE	NE	+	NE	NE	NE	NE	NE	++	NE	+	NE	NE	10	1	5	1
CCNU	-	++	-	NE	+	NE	-	NE	-	NE	-	NE	++	NE	+	NE	NE	8	5	2	2
MeCCNU	+	+	-	NE	-	NE	-	+	+	NE	-	NE	++	NE	+	-	NE	6	5	5	1
Streptozotocin	-	NE	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	2	0	0
DTIC	-	-	-	NE	NE	NE	NE	NE	-	NE	-	NE	NE	NE	++	+	NE	10	5	1	1
Hexamethylmelamine	+	++	+	NE	++	NE	NE	+	+	NE	-	NE	NE	NE	-	-	NE	8	3	4	2
Dibromodulcitol	-	+	+	NE	NE	NE	NE	NE	+	NE	+	NE	NE	NE	+	+	NE	10	1	6	0
Hydroxyurea	-	+	-	NE	NE	NE	NE	NE	+	NE	+	NE	NE	NE	-	NE	NE	11	3	3	0
Procarbazine	-	+	NE	NE	NE	NE	NE	NE	-	NE	NE	NE	+	NE	-	NE	NE	12	3	2	0
L-asparaginase	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
Dibromomannitol	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	17	0	0	0
5-Azacytidine	-	NE	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	2	0	0
DRUG ACTIVITY Spectrum of Tumors																					
NE	5	9	6	27	21	28	22	20	14	28	22	29	22	24	7	19	30				
Total = 30 -	18	9	11	1	2	1	2	0	4	0	6	0	1	0	17	3	0				
+	5	6	5	1	1	1	4	8	10	1	2	1	4	2	5	4	0				
++	2	6	8	1	6	0	2	2	2	1	0	0	3	4	1	4	0				

* NE = Not evaluated

- = Adequate evaluation; drug inactive

+ = Adequate evaluation; evidence of drug activity, but not clearly established

++ = Adequate evaluation; drug definitely active

Table 16. Background data for clinical evaluations.

DRUG	TUMOR TYPES																
	Colon	Lung	Breast	Pancreas	Ovary	Prostate	Stomach	Cervix	Head & Neck	Bladder	Kidney	Esophagus	Brain	Testicle	Melanoma	Sarcoma	Uterus
ALKYLATING AGENTS																	
Cyclophosphamide	13/71 (21%)	187/814 (23%)	182/525 (36%)	1/5 -	115/262 (44%)	5/13 -	0/4 -	36/187 (19%)	28/77 (36%)	2/10 -	2/12 -	–	8/14 -	2/2 -	9/55 (16%)	48/93 (52%)	4/16 (25%)
Nitrogen Mustard	6/50 (12%)	454/1264 (36%)	32/92 (35%)	1/7 -	0/8 -	2/8 -	4/25 (16%)	0/12 -	5/66 (8%)	–	–	–	–	–	3/45 (7%)	–	–
Chlorambucil	5/55 (9%)	1/21 (5%)	11/56 (20%)	0/6 -	200/390 (51%)	–	3/18 (17%)	11/44 (25%)	5/34 (15%)	0/18 -	2/15 -	–	–	6/8 -	2/22 (9%)	7/28 (25%)	0/11 -
Melphalan	19/110 (17%)	0/2 -	20/86 (23%)	–	173/366 (47%)	–	–	4/20 (20%)	–	–	–	–	–	49/86 (57%)	8/52 (15%)	–	–
Busulfan	1/10 -	15/169 (9%)	0/8 -	–	–	1/16 (6%)	–	–	–	–	–	–	–	–	1/10 -	–	–
ANTI-METABOLITES																	
5-Fluorouracil	454/2107 (21%)	11/157 (7%)	324/1263 (26%)	60/212 (28%)	32/101 (32%)	9/24 (38%)	107/414 (26%)	37/159 (23%)	18/118 (15%)	26/74 (35%)	3/40 (8%)	3/18 (17%)	1/19 (5%)	0/15 -	1/43 (2%)	–	7/34 (20%)
Methotrexate	19/111 (17%)	99/395 (25%)	120/356 (34%)	–	–	–	0/2 -	5/24 (21%)	87/232 (38%)	–	–	–	35/65 (54%)	0/10 -	2/25 (8%)	–	–
6-Mercaptopurine	2/50 (4%)	3/103 (3%)	6/45 (13%)	–	–	–	–	–	6/49 (12%)	–	1/15 -	–	–	–	2/37 (5%)	–	–
6-Thioguanine	1/35 (3%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ara-C	13/137 (10%)	0/43 -	6/64 (9%)	–	–	–	5/6 -	–	–	–	–	–	–	–	1/52 (2%)	–	–
MITOTIC INHIBITORS																	
Vincristine	0/26 -	2/37 (5%)	47/226 (20%)	–	0/17 -	1/10 -	–	10/34 (29%)	–	–	–	–	33/58 (57%)	0/7 -	6/52 (12%)	25/53 (47%)	–
Vinblastine	6/64 (9%)	17/151 (11%)	19/95 (20%)	–	3/20 (15%)	–	–	–	10/35 (29%)	–	1/12 -	–	21/48 (44%)	17/33 (52%)	11/71 (15%)	–	–
ANTITUMOR ANTIBIOTICS																	
Actinomycin D	7/48 (15%)	0/16 -	5/44 (11%)	0/4 -	–	–	–	–	–	–	–	–	–	16/31 (52%)	19/55 (35%)	20/83 (24%)	–
Mithramycin	4/28 (14%)	4/46 (9%)	14/70 (20%)	0/4 -	–	1/11 -	0/2 -	–	–	–	–	–	21/41 (52%)	155/431 (36%)	9/79 (11%)	2/39 (5%)	–
Daunomycin	0/9 -	1/14 -	0/2 -	–	–	–	–	–	–	–	–	–	–	–	0/6 -	–	–
Adriamycin	8/92 (9%)	53/314 (17%)	67/193 (35%)	–	18/48 (38%)	2/9 -	2/17 (12%)	9/28 (32%)	13/67 (19%)	27/97 (28%)	4/56 (7%)	–	–	12/60 (20%)	0/19 -	88/351 (25%)	2/6 -
Bleomycin	0/23 -	10/130 (8%)	3/26 (12%)	–	0/12 -	–	–	8/78 (10%)	49/158 (31%)	–	1/7 -	7/42 (17%)	15/21 (71%)	24/57 (42%)	0/14 -	–	–
Mitomycin C	35/219 (16%)	43/185 (23%)	41/110 (37%)	12/44 (27%)	–	0/2 -	58/185 (31%)	4/18 (22%)	2/11 -	–	4/36 (11%)	–	–	–	9/65 (14%)	18/55 (33%)	–
RANDOM SYNTHETICS AND MISCELLANEOUS																	
BCNU	17/128 (13%)	9/83 (11%)	16/76 (21%)	0/37 -	1/1 -	1/7 -	6/33 (18%)	1/7 -	4/25 (16%)	–	–	–	67/142 (47%)	2/8 -	22/122 (18%)	4/17 (24%)	–
CCNU	20/222 (9%)	46/262 (18%)	20/171 (12%)	3/19 (16%)	7/22 (32%)	–	1/35 (3%)	1/5 -	4/50 (8%)	–	4/59 (7%)	2/19 (10%)	47/115 (41%)	–	19/126 (15%)	1/22 (5%)	–
MeCCNU	18/148 (12%)	29/289 (10%)	3/93 (3%)	0/13 -	0/19 -	2/19 (11%)	1/28 (4%)	4/24 (17%)	6/40 (15%)	–	0/21 -	–	13/40 (33%)	0/4 -	15/119 (13%)	4/72 (6%)	–
Streptozotocin	8/85 (9%)	2/5 -	2/19 (11%)	3/27 (11%)	0/9 -	–	0/4 -	–	2/7 -	–	0/18 -	–	–	0/2 -	0/3 -	1/7 -	–
DTIC	7/84 (8%)	13/109 (12%)	5/51 (10%)	0/10 -	0/9 -	0/6 -	2/15 -	3/12 -	1/24 (5%)	0/7 -	1/29 (3%)	0/3 -	2/5 -	0/4 -	209/838 (25%)	15/127 (12%)	–
Hexamethylmelamine	11/95 (12%)	74/372 (20%)	11/54 (20%)	0/6 -	12/32 (38%)	2/6 -	2/12 -	5/17 (29%)	9/75 (12%)	3/10 -	1/17 (6%)	1/3 -	–	–	2/42 (5%)	1/43 (2%)	–
Dibromodulcitol	4/42 (10%)	37/255 (15%)	20/53 (37%)	–	1/8 -	1/3 -	3/8 -	–	11/50 (22%)	0/4 -	4/19 (21%)	0/1 -	–	0/1 -	8/42 (19%)	7/44 (16%)	–
Hydroxyurea	16/151 (10%)	10/84 (12%)	4/21 (19%)	–	–	–	6/31 (19%)	2/13 -	7/18 (39%)	5/8 -	7/25 (28%)	–	5/10 -	–	29/127 (23%)	–	–
Procarbazine	0/35 -	29/159 (18%)	1/21 (5%)	–	–	–	–	–	3/31 (10%)	1/3 -	–	–	14/27 (52%)	1/3 -	8/50 (16%)	–	–
L-Asparaginase	0/6 -	0/1 -	0/1 -	–	–	–	–	–	–	–	0/1 -	–	–	–	1/5 -	0/1 -	–
Dibromomannitol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5-Azacytidine	5/52 (10%)	0/9 -	11/53 (21%)	1/2 -	1/4 -	–	0/3 -	–	1/5 -	–	0/2 -	–	1/1 -	0/2 -	3/16 (19%)	0/4 -	–

Table 17. Ranking of tumors on the basis of the percentage of drug adequately evaluated.

Rank	Tumor Type	No. of drugs (of 30) adequately evaluated*	%
1	Colon	25	83
2	Breast	24	80
3	Melanoma	23	77
4	Lung	21	70
5	Head and Neck	16	53
6	Sarcoma	11	37
7	Cervix	10	33
8	Ovary	9	30
9	Brain	8	27
10	Kidney	8	27
11	Stomach	8	27
12	Testicle	6	20
13	Pancreas	3	10
14	Prostate	2	7
15	Bladder	2	7
16	Esophagus	1	3
17	Uterus	0	-

*Includes only drugs rated -, +, and ++ in Table 15.

Table 18. Ranking of tumors by percentage of drugs showing definite activity.

Rank	Tumor Type	No. drugs with definite activity* / No. drugs adequately evaluated	%
1	Testicle	4/6	66
2	Ovary	6/9	66
3	Bladder	1/2	50
4	Brain	3/8	38
5	Sarcoma	4/11	36
6	Breast	8/24	33
7	Pancreas	1/3	33
8	Lung	6/21	29
9	Stomach	2/8	25
10	Cervix	2/10	20
11	Head and Neck	2/16	13
12	Colon	2/25	8
13	Melanoma	1/23	4
14	Kidney	0/8	-
15	Prostate	0/2	-
16	Esophagus	0/1	-
17	Uterus	0/0	-

*Includes drugs rated ++ in Table 15.

Table 19. Ranking of tumors by percentage of drugs showing definite activity or evidence of activity

Rank	Tumor Type	No. of drugs with evidence of activity or definite activity* / No. drugs adequately evaluated	%
1	Cervix	10/10	100
2	Testicle	6/6	100
3	Bladder	2/2	100
4	Esophagus	1/1	100
5	Brain	7/8	88
6	Ovary	7/9	78
7	Head and Neck	12/16	75
8	Stomach	6/8	75
9	Sarcoma	8/11	73
10	Pancreas	2/3	66
11	Lung	12/21	60
12	Breast	13/24	54
13	Prostate	1/2	50
14	Colon	7/25	28
15	Melanoma	6/23	26
16	Kidney	2/8	25
17	Uterus	0/0	-

* Includes drugs rated + and ++ in Table 15.

Table 20. Ranking of drugs by percentage of tumors in which they have been adequately evaluated.

Rank	Drug	No. of tumors adequately evaluated* / Total Tumors (17)	%
1	5-Fluorouracil	12	70
2	Adriamycin	12	70
3	MeCCNU	11	65
4	Hexamethylmelamine	9	53
5	CCNU	9	53
6	Cyclophosphamide	8	47
7	Chlorambucil	8	47
8	Vincristine	8	47
9	Bleomycin	8	47
10	Methotrexate	7	41
11	Vinblastine	7	41
12	BCNU	7	41
13	Mitomycin C	7	41
14	DTIC	7	41
15	Dibromodulcitol	7	41
16	Melphalan	6	35
17	Nitrogen Mustard	6	35
18	Hydroxyurea	6	35
19	Mithramycin	6	35
20	Actinomycin D	5	30
21	Procarbazine	5	30
22	6-Mercaptopurine	5	30
23	Ara-C	4	24
24	Streptozotocin	2	12
25	Busulfan	2	12
26	5-Azacytidine	2	12
27	6-Thioguanine	1	9
28	L-asparaginase	0	-
29	Daunomycin	0	-
30	Dibromomannitol	0	-

*Includes drugs rated -, +, and ++ in Table 15.

Table 21. Ranking of drugs based on the number of tumors against which they are definitely active.

Rank	Drug	No. tumors showing definite response* / No. tumors adequately evaluated	%
1	Cyclophosphamide	5/8	63
2	5-Fluorouracil	6/12	50
3	Methotrexate	3/7	43
4	Mitomycin C	3/7	43
5	Adriamycin	5/12	42
6	Actinomycin D	2/5	40
7	Melphalan	2/6	33
8	Vinblastine	2/7	29
9	Vincristine	2/8	25
10	Bleomycin	2/8	25
11	CCNU	2/9	22
12	Hexamethylmelamine	2/9	22
13	Nitrogen mustard	1/6	17
14	Mithramycin	1/6	17
15	BCNU	1/7	14
16	DTIC	1/7	14
17	Chlorambucil	1/8	13
18	MeCCNU	1/11	9
19	Dibromodulcitol	0/7	-
20	Procarbazine	0/5	-
21	6-Mercaptopurine	0/5	-
22	Hydroxyurea	0/6	-
23	Ara-C	0/4	-
24	Busulfan	0/2	-
25	Streptozotocin	0/2	-
26	5-Azacytidine	0/2	-
27	6-Thioguanine	0/1	-
28	Daunomycin	0/0	-
29	L-asparaginase	0/0	-
30	Dibromomannitol	0/0	-

*Includes tumors ranked ++ in Table 15.

Table 22. Ranking of drugs based on the number of tumors against which they are definitely active or show evidence of activity.

Rank	Drug	No. tumors showing evidence of response and definite response* / No. tumors adequately evaluated	%
1	Cyclophosphamide	7/8	88
2	Methotrexate	6/7	86
3	Mitomycin C	6/7	86
4	BCNU	6/7	86
5	Dibromodulcitol	6/7	86
6	5-Fluorouracil	9/12	75
7	Adriamycin	9/12	75
8	Chlorambucil	6/8	75
9	Melphalan	4/6	66
10	Hexamethylmelamine	6/9	66
11	Actinomycin D	3/5	60
12	Vinblastine	4/7	57
13	MeCCNU	6/11	55
14	Vincristine	4/8	50
15	Bleomycin	4/8	50
16	Hydroxyurea	3/6	50
17	Nitrogen mustard	3/6	50
18	CCNU	4/9	44
19	Procarbazine	2/5	40
20	DTIC	2/7	29
21	6-Mercaptopurine	1/5	20
22	Mithramycin	1/6	17
23	Ara-C	0/4	-
24	Busulfan	0/2	-
25	Streptozotocin	0/2	-
26	5-Azacytidine	0/2	-
27	6-Thioguanine	0/1	-
28	Daunomycin	0/0	-
29	L-asparaginase	0/0	-
30	Dibromomannitol	0/0	-

*Includes tumors rated + and ++ in Table 15.

Table 23. Combined modality trials currently in progress.

Tumor type	Overall trials	Regimens *			
		Chemorx.	Immunorx.	Chemorx. + Immunorx.	RT + Chemorx.
Colon	4	1. 5-FU 2. MeCCNU+5-FU 3. MeCCNU+5-FU +VCR	1. MER BCG 2. BCG	1. MeCCNU+5-FU+ VCR+MER BCG 2. 5-FU+BCG	—
Pancreas	4	1. 5-FU 2. CCNU+5-FU	—	—	1. 4000R+5-FU 2. 6000R+5-FU
Stomach	3	1. 5-FU 2. MeCCNU+5-FU(2)	—	—	5000R+5-FU → 5-FU+MeCCNU
Breast	8	1. L-PAM (5) 2. L-PAM+5-FU 3. "CMF" (2) 4. "CFP" 5. "CMFV" 6. "CMF"+Tamoxifen 7. "CMFVP"		1. "CMF"+MER BCG 2. "CMF"+BCG (2) 3. "CMF"+BCG+ Allogeneic cells	RT + "CFP"
Lung (epidermoid)	4	1. CCNU 2. CCNU+hydroxyurea (2)	BCG	—	RT + CCNU + hydroxyurea
Lung (small cell)	4	1. CCNU+hydroxyurea 2. CCNU + CTX + MTX	BCG	—	1. RT+CCNU+ hydroxyurea 2. RT+CCNU+ CTX+MTX

(Table 23 - continued)

Melanoma	11	1. DTIC (4) 2. MeCCNU	1. BCG (6) 2. BCG+allogeneic cells (3) 3. *C. parvum* 4. BCG+"unblocking" plasma 5. BCG+"normal" plasma	1. DTIC+BCG(2) 2. DTIC+BCG+ allogeneic cells	—
Ovary	2	L-PAM (2)	—	—	RT+L-PAM
Cervix	1	—	—	—	RT+hydroxyurea
Brain (glioma)	3	1. BCNU 2. MeCCNU	—	—	1. RT+BCNU(3) 2. RT+CCNU 3. RT+MeCCNU
Testicular	2	1. Actinomycin D +Chlorambucil 2. VLB+Bleomycin	—	—	RT+Actinomycin D+Chlorambucil
Esophagus	2	—	—	—	1. RT+Bleomycin (2) 2. RT+hydroxyurea
Total	48	25	9	7	15

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*Numbers in parentheses refer to cases of 2 or more trials in progress with the same regimen.

APPENDIX A

Disease Oriented Review of Single Agent Data, Active Combinations, and Current Combined Modality Trials in Solid Tumors

Under section C (Combined Modality Trials) for each tumor category, the abbreviation in parenthesis refers to the performing group:

COG - Central Oncology Group
VASAG - VA Surgical Adjuvant Study Group
GITSG - GI Tumor Study Group
ECOG - Eastern Cooperative Study Group
LCWP - Lung Cancer Working Party
VALCSG - VA Lung Cancer Study Group
NSABG - National Surgical Adjuvant Breast Group
ALGB - Acute Leukemia Group B
GOG - Gynecologic Oncology Group
BTSG - Brain Tumor Study Group
TTSG - Testicular Tumor Study Group
WHOM - World Health Organization Melanoma Group
SEOG - Southeast Oncology Group

I. Large Bowel Cancer

A. Active single agents

1. 5-FU (21%)
2. MeCCNU (12%)
3. Mitomycin C (16%)

B. Active combinations

1. 5-FU + MeCCNU (30%)
2. 5-FU + MeCCNU + VCR (43%)

C. Combined modality trials (Dukes C lesions. Standard therapy - "curative" resection)

1. 5-FU *vs* Surgery (COG)
2. MeCCNU + 5-FU *vs* Surgery (VASAG)
3. MeCCNU + 5-FU + VCR *vs* MeCCNU + 5-FU + VCR + MER BCG *vs* MER BCG *vs* Surgery (GITSG)
4. 5-FU + BCG *vs* BCG (M. D. Anderson, Dept. of Developmental Therapeutics)

II. Pancreatic Cancer

A. Active single agents

5-FU (28%)

B. Active combinations

None

C. Combined modality trials

1. Localized disease. Standard therapy - Whipple procedure or total pancreatectomy

   Surgery + RT + 5-FU vs Surgery (GITSG)

2. Regional disease, locally unresectable for cure. Standard therapy - palliative bypass surgery

   a. Surgery + 4000R + 5-FU vs Surgery + 6000R + 5-FU vs Surgery + 6000R (GITSG)

   b. Surgery + CCNU + 5-FU vs Surgery (VASAG)

   c. Surgery + RT + 5-FU vs Surgery + 5-FU (ECOG)

III. Stomach Cancer

A. Active single agents

1. 5-FU (26%)
2. Mitomycin C (31%)
3. BCNU (18%)

B. Active combinations

1. 5-FU + MeCCCNU (52%)
2. 5-FU + BCNU (40-45%)

C. Combined modality trials

1. Localized Stage II-III. Standard therapy - curative resection

   a. Surgery + MeCCNU + 5-FU vs Surgery (GITSG)

   b. Surgery + MeCCNU + 5-FU vs Surgery (VASAG)

2. Stage III, locally unresectable.

   Surgery + MeCCNU + 5-FU vs Surgery + RT + 5-FU
   MeCCNU + 5-FU (GITSG)

IV. Breast Cancer

A. Active single agents

1. Adriamycin (37%)
2. CTX (34%)
3. 5-FU (26%)
4. MTX (34%)
5. L-PAM (20%)
6. VCR (20%)
7. VLB (20%)
8. Mitomycin C (37%)

B. Active combinations

1. "CMF" - CTX + 5-FU + MTX (53%)
2. "CFP" - CTX + 5-FU + Prednisone (52%)
3. "CMFV" - CTX + 5-FU + MTX + VCR (49%)
4. "CMFVP" - CTX + 5-FU + MTX + VCR + Prednisone (50-59%)
5. CTX + Adriamycin (70-80%)
6. Adriamycin + VCR (50-53%)

C. Combined modality trials (Stage II disease. Standard therapy - radical or modified radical mastectomy)

1. L-PAM vs Control (NSABG, ECOG)
2. L-PAM + 5-FU vs L-PAM (NSABG, ECOG)
3. "CMF" vs Control (NCI, Milan)
4. "CFP" vs "CFP" + RT vs L-PAM (Mayo Clinic)
5. "CMF" vs "CMF" + MER BCG vs "CMFVP" (ALGB)

6. "CMF" + BCG vs "CMF" + Tamoxifen vs L-PAM (Case Western Reserve)

7. "CMF" + BCG vs "CMF" + BCG + Allogeneic cells (UCLA)

8. "CMFV" vs L-PAM (COG)

V. Lung Cancer (Epidermoid)

A. Active single agents (none have significant impact on survival)

1. MTX (25%)

2. Nitrogen mustard (21%)

3. CTX (20%)

4. Adriamycin (19%)

B. Active combinations

None

C. Combined modality trials

1. Stage I & II. Standard therapy - lobectomy or pneumonectomy

a. Surgery + CCNU * vs Surgery (LCWP)

b. Surgery + CCNU + hydroxyurea† vs Surgery (VASAG)

c. Surgery + BCG vs Surgery (ECOG)

2. Stage III "regional disease". Standard therapy - RT

RT + CCNU + hydroxyurea vs RT (VALCSG)

VI. Lung cancer (Small cell anaplastic)

A. Active single agents (none have significant impact on survival)

1. MTX (39%)

2. Nitrogen mustard (39%)

3. CTX (31%)

4. Hexamethylmelamine (26%)

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*CCNU 17% response rate
†No data specifically for epidermoid carcinoma

    5. Adriamycin (25%)
    6. Procarbazine (25%)
    7. BCNU (21%)
    8. MeCCNU (21%)
    9. CCNU (19%)

B. Active combinations

    1. As defined by increased survival over single agents - None
    2. By objective response rate
        a. CCNU + CTX
        b. CCNU + CTX + MTX

C. Combined modality trials

    1. Stage III* - "Local" disease. Standard therapy - RT or surgery
        a. Surgery + CCNU + hydroxyurea vs Surgery (VASAG)
        b. Surgery + BCG vs Surgery (ECOG)
    2. Stage III* - "Regional" disease. Standard therapy - RT
        a. RT + CCNU + CTX + MTX vs same therapy + prophylactic brain irradiation (LCWP)
        b. RT + CCNU + hydroxyurea vs RT (VALCSG)

/II. Malignant Melanoma

A. Active single agents

    DTIC (25%)

B. Active combinations

    None

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*All small cell carcinoma is effectively Stage III

C. Combined modality trials (Variable stage I-III. Standard therapy - wide surgical excision ± lymph node dissection)

1. Stage I, level III, IV, V; Stage II, extremity/head & neck, with excised local and/or nodal recurrence

   Surgery + BCG vs Surgery (ECOG)

2. Stage II with recurrence within 5 cm of 1° or in adjacent nodes, after excision of all nodes

   Surgery + DTIC vs Surgery + BCG (ECOG)

3. Stage I (poor prognosis), Stage II-III (all known tumor excised)

   Surgery + DTIC vs Surgery (COG)

4. Stage II

   Surgery + MeCCNU vs Surgery + BCG + allogeneic cells vs Surgery (NCI)

5. Stage I (trunk node dissection), Stage II (clinical), all other sites

   Surgery + DTIC vs Surgery + BCG vs Surgery + DTIC + BCG vs Surgery (WHOM)

6. Stage I trunk, no demonstrable disease after surgery

   Surgery + BCG vs Surgery (M. D. Anderson, Dept. of Developmental Therapeutics)

7. Stage III regional node metastasis $\geq$ 5 positive nodes with no demonstrable disease after surgery, Stage IV

   Surgery + DTIC + BCG vs Surgery + BCG (M. D. Anderson, Dept. of Developmental Therapeutics)

8. Stage I, level III-V or vascular invasion, Stage II

   Surgery + BCG + Allogeneic cells vs Surgery + BCG vs Surgery (UCLA)

9. Stage I (poor prognosis), Stage II-III

   Surgery + DTIC + BCG + allogeneic cells vs Surgery + DTIC vs Surgery + BCG + Allogeneic cells vs Surgery (U. Minnesota)

10. Stage II-III

Surgery + BCG + Plasma with "unblocking" activity *vs* Surgery + BCG + "normal" plasma *vs* Surgery (U. Washington)

11. Stage I-II

Surgery + *C. parvum* *vs* Surgery (SEOG)

VIII. Ovarian Cancer

A. Active single agents

1. L-PAM (47%)

2. CTX (44%)

3. Hexamethylmelamine (38%)

4. Adriamycin (38%)

5. 5-FU (32%)

6. Chlorambucil (51%)

B. Active combinations

None

C. Combined modality trials.

1. Stage I. Standard therapy - surgery.

Surgery + L-PAM *vs* Surgery + RT *vs* Surgery (GOG)

2. Stage III. Standard therapy - RT

Surgery and randomization to RT *vs* RT   L-PAM *vs* L-PAM RT *vs* L-PAM (GOG)

IX. Cervical Cancer

A. Active single agents

1. CTX (19%)

2. 5-FU (23%)

B. Active combinations

None

C. Combined modality trials (Stage IIIb, IV confined to pelvis. Standard therapy - RT)

RT + hydroxyurea vs RT (GOG)

X. Brain Tumors (Malignant glioma)

A. Active single agents

1. BCNU (47%)
2. CCNU (41%)
3. MeCCNU (33%)
4. Procarbazine (52%)

B. Active combinations

None

C. Combined modality trials (locally unresectable disease. Standard therapy - surgical decompression and RT)

1. RT vs BCNU vs RT + BCNU vs Control (BTSG)
2. RT vs RT + BCNU vsRT + MeCCNU vs MeCCNU (BTSG)
3. RT vs RT + BCNU vs RT + CCNU (NCI, Milan)

XI. Testicular Cancer

A. Active single agents

1. VLB (52%)
2. Actinomycin D (52%)
3. Bleomycin (42%)
4. Adriamycin (20%)
5. Mithramycin (36%)
6. L-PAM (57%)

B. Active combinations

VLB + Bleomycin (≃ 90%)

C. Combined modality trials (Stage II non-seminomatous disease. Standard therapy - surgery)

1. Surgery + RT vs Surgery + Actinomycin D + Chlorambucil vs Surgery + RT + Actinomycin D + Chlorambucil (TTSG)

2. Surgery + VLB + Bleomycin (M. D. Anderson)

XII. Esophageal Cancer

A. Active single agents

Bleomycin (17%)

B. Active combinatons

None

C. Combined modality trials (Stage III. Standard therapy - surgical bypass or RT)

1. RT (5000-6000 R) vs RT + Bleomycin (ECOG)

2. RT vs RT + Bleomycin vs RT + hydroxyurea (VASAG)

XIII. Bladder Cancer

A. Active single agents

Adriamycin (28%)

B. Active combinations

None

C. Combined modality trials

None

XIV. Head and Neck Cancer

A. Active single agents

1. MTX (38%)

2. Bleomycin (31%)

3. CTX (36%)

4. 5-FU (15%)

B. Active combinations

None

C. Combined modality trials

None

XV. Renal Cancer

A. Active single agents

Provera (15-20%)

B. Active combinations

None

C. Combined modality trials

None

XVI. Prostate

A. Active single agents

None

B. Active combinations

None

C. Combined modality trials

None

XVII. Endometrial Cancer

A. Active single agents

None

B. Active combinations

None

C. Combined modality trials

None

XVIII. Sarcomas (general)

A. Active single agents

1. Adriamycin (25%)

    2. Actinomycin D (24%)
    3. CTX (52%)
    4. VCR (47%)

B. Active combinations

    1. Adriamycin + DTIC (40-45%)
    2. Adriamycin + DTIC + CTX + VCR (60-70%)

C. Combined modality trials*

None

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*Excluding osteogenic sarcoma, Ewings sarcoma, and embryonal rhabdomyosarcoma

LITERATURE RESEARCH SECTION

The Literature Research Section serves the Division of Cancer Treatment staff by providing information from published literature on all aspects of the therapy of cancer. The staff uses data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy and related chemical and biomedical disciplines in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluating toxicological and clinical studies.

Literature Services. The Section received and filled over 200 requests for information during the year. Responses were provided as comprehensive or selective bibliographies, computer print-outs, abstracts and copies of articles. Over half of the requests entailed manual literature searches supplemented by various automated bibliographic retrieval systems such as Medline, Toxline, and Cancerline. Extensive clinical searches were performed for various modalities of treatment of hepatoma, neoplasms of the colon and rectum, and long-term remissions in leukemia. Bibliographies were prepared or updated for such agents as bleomycin, L-asparaginase, nafoxidine, olivomycin, chlorozotocin, BCG, methyl CCNU and pyrazofurin. A recurring bibliography on adverse effects of antitumor agents which NCI has in the clinic continues to prepared.

The Section coordinates NCI access to the on-line data bases of the Medlars automated bibliographic search system. In this role the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. It is also the center for access to the Toxline and Cancerline data bases for personnel in the Blair Building. These computer services aid in searching and facilitate answering of requests. This year, responses to more than 50 requests were in the form of compouter print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

The staff continues to be responsible for the preparation and distribution of the agent folders for Decision Network meetings. Each folder contains the structure and systematic name of the compound, an analog search, a bibliography from the published literature, summaries of screening, toxicological or other preclinical or clinical data, and questionnaires. Materials were assembled for a total of 32 drugs during the year.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books, journals and films for the use of NCI staff. Over 1,000 journals were circulated during the year and approximately $15,000 was spent on books and journal subscriptions. Near the end of the year a second library in the Blair Building became operational. These holdings consist of some of the cancer journals, abstracting and indexing secondary sources, and other journals of special interest to the personnel in the building.

Contract Operations. Contracts for the preparation of Cancer Chemotherapy Abstracts have been monitored by members of the Literature

Research Section since 1965. To reflect the broader interest of the DCT, the journal coverage was expanded to include surgery, radiotherapy and immunotherapy of cancer. The first issue of Cancer Therapy Abstracts, was published during 1974. In addition to the printed journal and hard copy of all cited articles, the Section receives computer tapes of the abstracts and indexes. Under the sponsorship of the Office of Program Planning and Analysis, these machine readable records since 1967 constitute the input to the Cancerline data base which can be searched on-line through the Medlars network.

COMBINED MODALITY BRANCH

Current Status of Contract Supported Groups

Testicular Tumor Study Group. In an attempt to foster studies of intensive combined modality approaches in treating patients with non-seminomatous testicular tumors, this group was formed with Dr. Robert Golbey as chairman and administrative support by the Cancer Therapy Evaluation Program. The group has initiated a Stage II disease protocol which has begun to accrue patient entry and also has proposed a protocol for Stage III disease using an induction and maintenance regimen of combination chemotherapy. The active protocol for adjuvant therapy in Stage II disease is:

a) No gross residual disease: Radiation (abdominal + mediastinal) versus Vinblastine (5 mg/m^2/wk x 6) + Actinomycin D (0.5 mg/m^2 x 6) + Bleomycin (15 mg/m^2 twice weekly x 6 to total 300 mg)

b) Gross residual disease: Radiation ± chemotherapy (as above)

Gastrointestinal Tumor Study Group. Protocols for pancreatic and gastric cancer have been implemented and have begun patient accrual. RFPs are currently being evaluated for combined modality studies in colo-rectal carcinoma, which will include adjuvant studies and treatment arms for radiotherapy alone or combined with chemotherapy or immunotherapy.

Pancreas protocols are as follows:

a) Advanced carcinoma: Adriamycin (60 mg/m^2 IV q 3 wks to total 500 mg/m^2) versus Methotrexate (40 mg/m^2/wk IV) versus Actinomycin D (0.65 mg/m^2/d x 5 IV q 28 d)

b) Locally unresectable carcinoma: Radiotherapy (6000R) versus Radiotherapy (4000R) + 5-FU (500 mg/m^2/d x 3) followed by 5-FU (500 mg/m^2/wk) beginning 4 wks after completion of radiotherapy versus Radiotherapy (6000R) + chemotherapy (as above).

Gastric protocols include:

a) Adjuvant chemotherapy after curative resection: No treatment versus 5-FU (400 mg/m^2/d x 5 IV q 5 wks) + BCNU (40 mg/m^2/d x 5 IV q 10 wks)

b) Locally advanced surgically incurable carcinoma: 5-FU (325 mg/m^2/d x 5 IV) + MeCCNU (150 mg/m^2 PO q 10 wks) versus Radiotherapy (5000R split course) + 5-FU (500 mg/m^2/d x 3) with each RT course, followed by 5-FU + MeCCNU as above.

c) Advanced gastric carcinoma: Adriamycin (60 mg/m^2 IV q 3 wks) versus 5-FU (350 mg/m^2/d x 5 IV q 5 wks) + MeCCNU (150 mg/m^2 PO q 5 wks) + Adriamycin (40 mg/m^2 IV q 5 wks) versus 5-FU (300 mg/m^2/d x 5 IV q 5 wks) + Mitomycin C (1.5 mg/m^2/d x 5 q 5 wks) + Ara-C (50 mg/m^2/d x 5 q 5 wks)

Lung Cancer Working Party. This group was organized to plan and execute cooperative studies on the the therapy of lung cancer with single and combined modalities. The group chairman is Dr. Oleg Selawry and the Executive Officer is Dr. Ronald Vincent. Hopefully, current membership will be expanded from the present 6 institutions to 9 or 10, which will facilitate patient accrual and expediate progress of group studies.

Current active protocols are outlined in Figures 1 through 10.

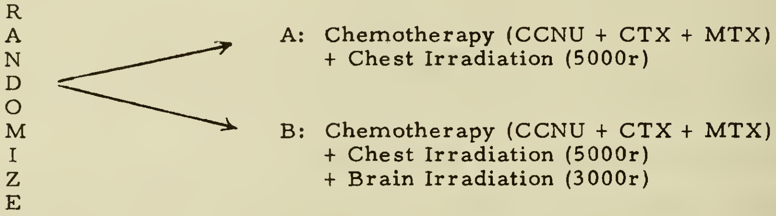

RANDOMIZE
A: Chemotherapy (CCNU + CTX + MTX)
+ Chest Irradiation (5000r)
B: Chemotherapy (CCNU + CTX + MTX)
+ Chest Irradiation (5000r)
+ Brain Irradiation (3000r)

Figure 1. Lung Cancer Working Party Protocol 7221 Intensive chemotherapy and radiotherapy of regional small cell carcinoma of the lung (H. Hansen & R. Sealy, Chairmen; S. Lagakos, Statistician).

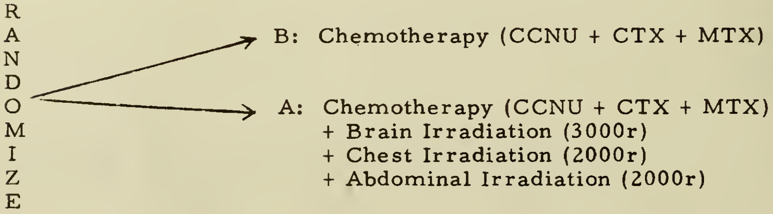
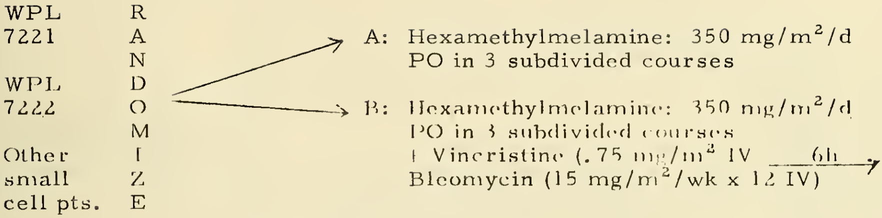

RANDOMIZE
B: Chemotherapy (CCNU + CTX + MTX)
A: Chemotherapy (CCNU + CTX + MTX)
+ Brain Irradiation (3000r)
+ Chest Irradiation (2000r)
+ Abdominal Irradiation (2000r)

Figure 2. Lung Cancer Working Party Protocol 7222 Intensive chemotherapy and radiotherapy of extensive small cell carcinoma of the lung (H. Hansen & R. Sealy, Chairmen; S. Lagakos, Statistician).



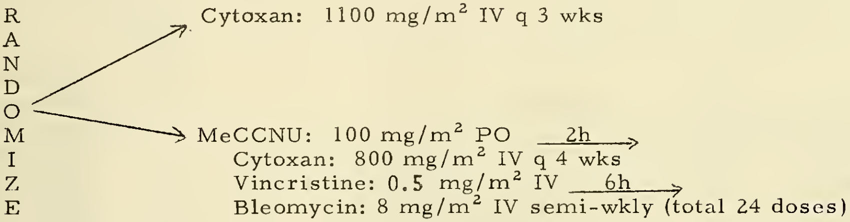
WPL 7221
WPL 7222
Other small cell pts.

RANDOMIZE

A: Hexamethylmelamine: 350 mg/m^2/d
PO in 3 subdivided courses

B: Hexamethylmelamine: 350 mg/m^2/d
PO in 3 subdivided courses
+ Vincristine (.75 mg/m^2 IV $\xrightarrow{6h}$
Bleomycin (15 mg/m^2/wk x 12 IV)

Figure 3. Lung Cancer Working Party Protocol 7223. Intensive chemotherapy of progressive small cell carcinoma of the lung (H. Hansen & R. Sealy, Chairmen; S. Lagakos, Statistician).

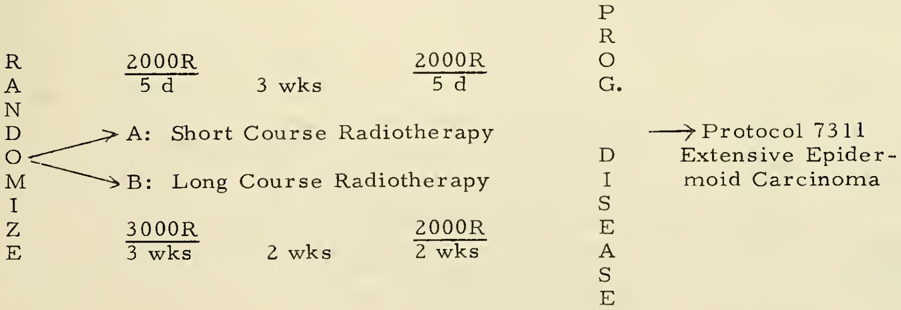


RANDOMIZE

Cytoxan: 1100 mg/m^2 IV q 3 wks

MeCCNU: 100 mg/m^2 PO $\xrightarrow{2h}$
Cytoxan: 800 mg/m^2 IV q 4 wks
Vincristine: 0.5 mg/m^2 IV $\xrightarrow{6h}$
Bleomycin: 8 mg/m^2 IV semi-wkly (total 24 doses)

Figure 4. Lung Cancer Working Party Protocol 7311. Chemotherapy of extensive epidermoid carcinoma of the lung (G. Bodey, Chairman; S. Lagakos, Statistician).



RANDOMIZE

$\frac{2000R}{5\ d}$ 3 wks $\frac{2000R}{5\ d}$

A: Short Course Radiotherapy

B: Long Course Radiotherapy

$\frac{3000R}{3\ wks}$ 2 wks $\frac{2000R}{2\ wks}$

PROG. DISEASE

→ Protocol 7311
Extensive Epidermoid Carcinoma

Figure 5. Lung Cancer Working Party Protocol 7312. Radiotherapy of regional epidermoid carcinoma of the lung (R. Sealy, Chairman; S. Lagakos, Statistician).

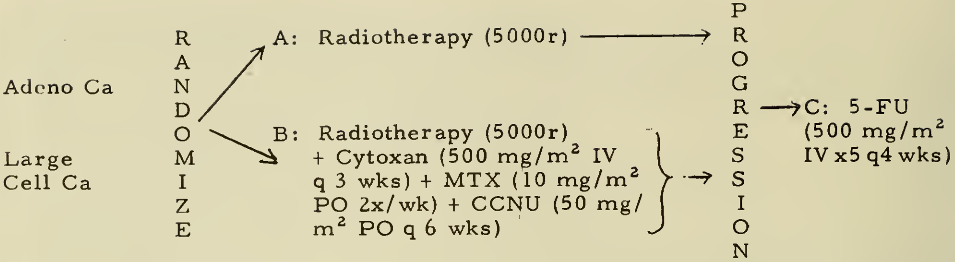

Adeno Ca
Large Cell Ca
RANDOMIZE
A: Radiotherapy (5000r)
B: Radiotherapy (5000r) + Cytoxan (500 mg/m² IV q 3 wks) + MTX (10 mg/m² PO 2x/wk) + CCNU (50 mg/m² PO q 6 wks)
PROGRESSION
C: 5-FU (500 mg/m² IV x5 q4 wks)

Figure 6. Lung Cancer Working Party Protocol 7331. Therapy of limited extent adenocarcioma and large cell carcinoma of the lung (D. T. Carr, Chairman; S. Lagakos, Statistician).

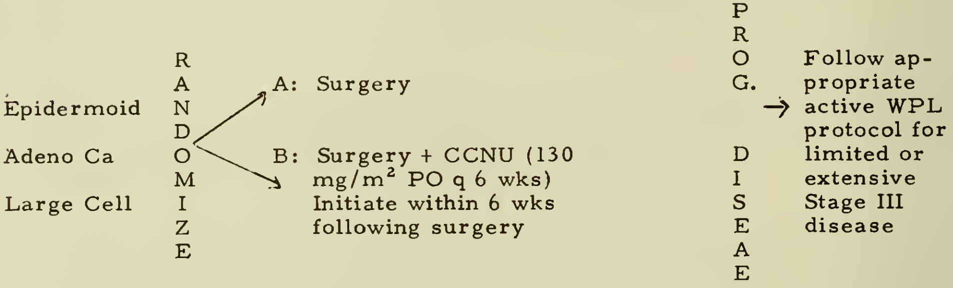

Epidermoid
Adeno Ca
Large Cell
RANDOMIZE
A: Surgery
B: Surgery + CCNU (130 mg/m² PO q 6 wks) Initiate within 6 wks following surgery
PROG. DISEAE
Follow appropriate active WPL protocol for limited or extensive Stage III disease

Figure 7. Lung Cancer working Party Protocol 7351. Therapy of completely resectable epidermoid, adenocarcinoma, and large cell carcinoma of lung (Stage I-II) (C. F. Mountain, Chairman).

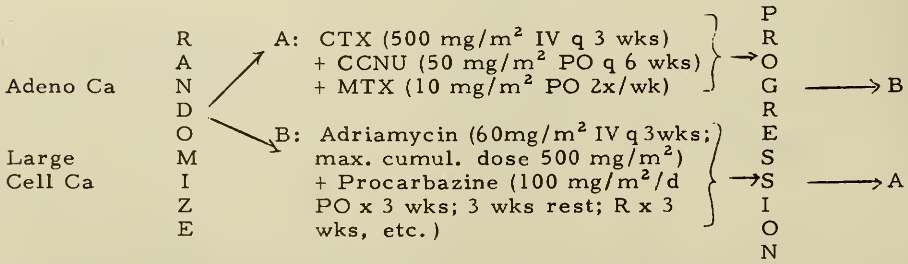

Adeno Ca
Large Cell Ca
RANDOMIZE
A: CTX (500 mg/m² IV q 3 wks) + CCNU (50 mg/m² PO q 6 wks) + MTX (10 mg/m² PO 2x/wk)
B: Adriamycin (60mg/m² IV q 3wks; max. cumul. dose 500 mg/m²) + Procarbazine (100 mg/m²/d PO x 3 wks; 3 wks rest; R x 3 wks, etc.)
PROGRESSION
B
A

Figure 8. Lung Cancer Working Party Protocol 7452. Combination chemotherapy of extensive adenocarcinoma and large cell carcinoma of the lung (A. Mittelman, Chairman; N. Sedransk, Statistician).

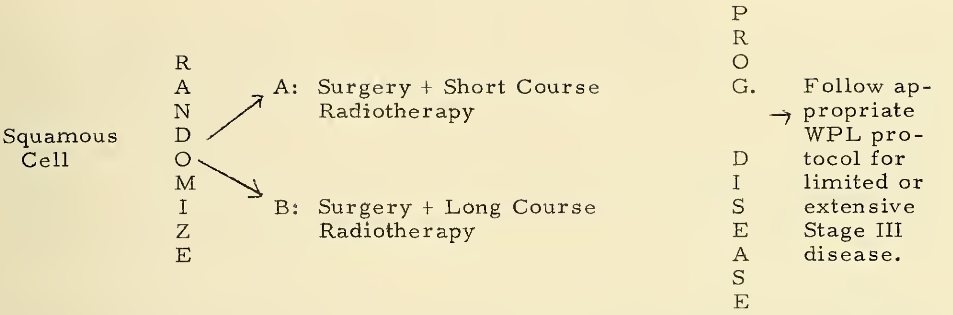

Squamous Cell
RANDOMIZE
A: Surgery + Short Course Radiotherapy
B: Surgery + Long Course Radiotherapy
PROG. DISEASE
Follow appropriate WPL protocol for limited or extensive Stage III disease.

Figure 9. Lung Cancer Working Party Protocol 7511. Adjuvant radiotherapy with adjunctive reductive surgery of Stage III squamous cell (epidermoid carcinoma of the lung (residual disease present) (T. E. Williams, Chairman).

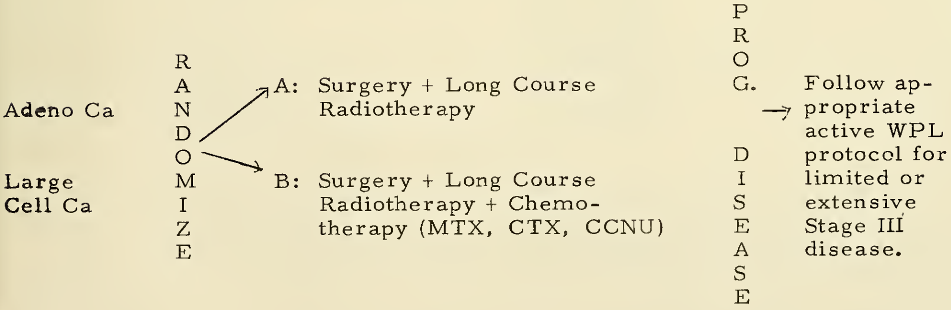

Adeno Ca
Large Cell Ca
RANDOMIZE
A: Surgery + Long Course Radiotherapy
B: Surgery + Long Course Radiotherapy + Chemotherapy (MTX, CTX, CCNU)
PROG. DISEASE
Follow appropriate active WPL protocol for limited or extensive Stage III disease.

Figure 10. Lung Cancer Working Party Protocol 7552. Adjuvant radiotherapy with adjunctive reductive surgery of Stage III adenocarcinoma and large cell carcinoma of the lung (residual disease present) (R. G. Vincent, Chairman).

In the past fiscal year the Investigational Drug Branch functioned in three major directions: (a) chemotherapy evaluation, (b) regulatory aspects, and (c) distribution of investigational drugs.

Organizationally the Branch consists of the Office of the Chief and the Drug Liaison and Distribution Section. The professional staff of the Branch includes the Physician, Pharmacologists, and Pharmacist and three staff associate Physicians, who spend the first year of their CTEP training in the Investigational Drug Branch.

The coordination of all functions of the Investigational Drug Branch is done by the Office of the Chief, which in addition is responsible for chemotherapy evaluation and liaison with cooperative groups, evaluation and approval of investigational drug protocols submitted by investigators conducting independent studies, and training of the staff associates in the drug oriented part of the CTEP. In addition the branch participated in a substantial number of international activities. The regulatory function and distribution of investigational drugs throughout the United States and the world is provided by the Drug Liaison and Distribution Section.

Phase I Studies: Fifteen investigational drugs sponsored by the DCT were studied in Phase I trials:

d-Tetrandrine	NSC 77037
Thalicarpine	NSC 68075
Cyclocytidine*	NSC 145668
α-TGdR	NSC 71851
Soluble Baker's Antifol*	NSC 139105
Cytembena*	NSC 104801
Piperazinedione*	NSC 135758
Diglycoaldehyde*	NSC 118994
Tritylcysteine*	NSC 83265
Asaley*	NSC 167780
Ftorafur*	NSC 198958
MER	NSC 143769
Gallium Nitrate	NSC 15200
Galactitol*	NSC 132313
Anguidine	NSC 141537

*Contract supported

The Phase I studies were conducted by members of the Phase I Working Group, which consists of clinical investigators from the NCI intramural program, clinical investigators from 7 institutions awarded the Phase I contract, and 4 institutions conducting Phase I studies without any financial support.

Thirty-two (32) Phase I studies were conducted, fifteen of them completed, and 10 Phase I drugs recently entered Phase II studies. Pharmacokinetic studies in man were included in 5 Phase I studies. The coordination of Phase I studies is accomplished by ad hoc telephone conferences and by Phase I

Working Group meetings four times a year. Detailed information on the current status of Phase I drugs has been summarized in the minutes of the last Phase I Working Group and is available from the Investigational Drug Branch.

Phase I-II Studies: Among the drugs evaluated in Phase II-III studies are:

5-Azacytidine	NSC 102816
Cis Platinum-diamminedichloride	NSC 119875
Chromomycin A_3	NSC 58514
DTIC	NSC 45388
Dibromodulcitol	NSC 104800
Hexamethylmelamine	NSC 13875
ICRF-159	NSC 129943
Isophosphamide	NSC 109724
BCNU	NSC 409962
CCNU	NSC 79037
MeCCNU	NSC 95441
Streptozotocin	NSC 85998
VM-26	NSC 122819
VP-16	NSC 141340

The drugs which became commercially available or for which new drug applications have been filed include:

Adriamycin	NSC 123127	(Adria Labs)
Mitomycin C	NSC 26988	(Bristol)
DTIC	NSC 45388	(Dome)
BCNU	NSC 409962	(Bristol)
CCNU	NSC 79037	(Bristol)

The update and exchange of information on Phase II-III clinical studies was accomplished in the New Drug Liaison Meeting, Febraury 25. Detailed information on these drugs is available from the Investigational Drug Branch.

Liaison with Cooperative Groups: Within these responsibilities the members of the professional staff attended more than 30 cooperative group meetings in the past year. This direct communication proved to be important and beneficial for all parties. Information from Investigational Drug Branch on current status of protocols and drug-disease oriented studies is actively required by the members of the groups. On the other hand, the flow of information from the cooperative groups to the Branch is smoother and not delayed until receipt of the minutes of the group meetings.

Evaluation of Independent Investigator Protocols: Within the last year an average of 15 protocols a week were submitted by independent investigators for approval; 978 new protocols were received, evaluated, and filed with the FDA. This represents a substantial work load as well as a moral and legal responsibility for the staff of the Branch. The protocol review committee, consisting of the professional staff of Investigational Drug Branch and Combined Modalities Branch meets weekly or biweekly in order to evaluate the scientific merit and relevance of the protocols. The committee is chaired by the Chief, Investigational Drug Branch.

Training of Staff Associates: In the last year three new associates joined the training program of the Branch. This consists of the drug-disease oriented program in cancer chemotherapy and clinical pharmacology of anticancer drugs. In this program, in addition to the daily educational activities, series of lectures on drug development, screening, mode of action, clinical pharmacology, toxicology and chemotherapy of leukemias, lymphomas, and solid tumors were given by the members of the CTEP, Drug Development, and Experimental Therapeutics staff. This program will be further expanded.

Drug Liaison and Distribution Section

The Section is responsible for compliance with FDA regulations regarding the use of investigational anticancer drugs in man, for supply and distribution of both investigational and commercially available drugs to various clinical investigators through the U. S. and many foreign countries, and for serving as an interface between various clinical investigators and NCI staff regarding any problems concerning drug purity, formulation, and stability.

The staff of the Section was increased in the past year by the addition of a pharmacist, Paul J. Vilk.

In compliance with FDA requirements, the following original Investigational New Drug Applications (IND) were submitted in Fiscal year 1974:

1. BCG Vaccine - Connaught (NSC B116341)
2. Corynebacterium Parvum (NSC 220537)
3. Dianhydrogalactitol (NSC 132313)
4. Spironolactone (NSC 150399)
5. Anguidine (NSC 141537)
6. Dichloroallyl Lawsone (NSC 126771)

These submissions bring the total of IND exemptions managed by the section to 125.

The section received a number of adverse drug reaction reports from clinical investigators. These reports were promptly brought to the attention of the FDA. When serious and life-threatening reactions were reported, the section alerted other investigators using the drug by sending warning letters giving details on the reaction.

The section staff periodically met with the FDA staff in the past year to discuss such matters as animal toxicology, clinical protocols or formulation problems.

Another primary activity of the section was the interfacing with Cooperative Study Groups and independent investigators. Through this interface, clinical drug needs for more than 200 dosage forms were planned and monitored. The section staff also attended cooperative study group meetings at which drug availability and regulatory information was discussed.

Routinely the section provided pharmacological and pharmaceutical input to clinical protocol design. The section provided liaison between the cooperative groups and NCI staff concerning drug related problems such as drug purity, stability and formulation.

An essential section function involves drug requests from investigators. Each request is reviewed to ascertain that Federal regulatory requirements are met. The request is then processed in an on-line computerized drug inventory system and drug distribution is authorized. In the past year 12,469 drug requests were processed.

Concurrent with these distribution activities, 656 new clinical investigators were registered and 978 new protocols were reviewed by the section.

In conjunction with the Chief of the Investigational Drug Branch, a clinical pharmacology liaison was established with the cooperative groups. Such information as drug metabolism, pharmacokinetics, and assay methods was provided. Pharmacological presentations were made to the Central Oncology Group for the drugs imidazole carboxamide (DTIC, NSC 45388) and cycloclucine (NSC 1026).

Liaison within NCI was facilitated through the section's participation in several committees.

1. Drug Status Committee - The section provided clinical input for the maintenance of adequate supplies of clinical drugs.
2. Operating Committee - Phase I clinical trial data and preclinical drug information was supplied by the Section. The section head functioned as the Executive Secretary of the Operating Committee.
3. Decision Network Committee - The section participated with NCI senior staff in all aspects of drug development. The section head functioned as Executive Secretary.
4. Drug Evaluation Committee - Animal screening data is studied and reviewed for potential clinical application.
5. Quality Control Committee - Section staff reviewed the potency and purity of all bulk and formulated anticancer drugs prior to their release for clinical use.
6. Protocol Review Committee - This committee is chaired by the Chief, Investigational Drug Branch. Clinical protocols submitted by independent investigators were reviewed by the staff of the section and pharmacological and pharmaceutical judgements were made.

Another section function is in the area of Investigational Drug Branch international affairs. The section head met with visitors from various foreign countries to discuss aspects of drug development, regulatory matters and their relationship to clinical trials.

Last year the section interacted with major pharmaceutical firms by giving assistance in the preparation of New Drug Applications (NDA) for the marketing of drugs evaluated in the Division of Cancer Treament. The following companies and drugs were involved:

1. Philips Roxane Laboratories - Hexamethylmelamine (NSC 13875)
2. Miles Laboratories - DTIC (NSC 45388)
3. Bristol Laboratories - BCNU, carmustine (NSC 409962)

4. Adria Laboratories - Doxorubicin (NSC 123127)

In summary, during the past fiscal year the responsibilities of Investigational Drug Branch increased. This resulted in both quantitative and qualitative increase of activities, including monitoring of clinical pharmacology studies, coordination of Phase I studies through the contract mechanism and Phase I working group, evaluation of Phase II-III studies, regulatory aspects, interface with cooperative study groups and independent investigators, drug authorization and distribution and international activities. In spite of the overload of administrative work the members of the professional staff increased their scientific activities, which resulted in significant increase in the number of presentations and publications.

HONORS AND AWARDS

1. Slavik, M. Elected to membership in The International Society for Chronobiology.
2. Slavik, M. Invited to Carlo ERBA Foundation Lecture, Milan, Italy, October 1974.
3. Slavik, M. Elected Chairman of the Section on Hematologic and Neoplastic Disease of the ASCPT.
4. Penta, J. S. Elected to membership of Clinical Pharmacology Subcommittee, Central Oncology Group, September 1974.
5. Legha, S. S. Certified Diplomate American College of Physicians, 1974.

PUBLICATIONS

1. Slavik, M. Investigational Drugs in Cancer Chemotherapy. Wadley Medical Bulletin 5:29-43, 1975.
2. Strauss, G. M., Penta, J. S., and Slavik, M.: Cyclocytidine (NSC 145668). Clinical Brochure, March 1975.
3. Legha, S. S. and Slavik, M. Comparative analysis of aureolic acid analogues. Clin. Pharm. Ther. 17:238-239, 1975.

PRESENTATIONS

1. Slavik, M.: Drug development and clinical testing of anticancer drugs. American Cancer Society Meeting, Bethesda, Md., April 17, 1974.
2. Carter, S. K. and Slavik, M.: A chemotherapist's perspectives on clinical trials with Corynebacterium Parvum. International Symposium on C. parvum, College de france, Paris, May 10, 1974.
3. Slavik, M.: Phase I study design and quantitative analysis of toxicity in man. St. Jedes Hospital, Memphis, Tennessee, May 15, 1974.

4. Slavik, M.: Overview on clinical data on butocin. Decision Network Meeting, Bethesda, Md., May 20, 1974.

5. Slavik, M.: New drugs available for Phase I studies. Phase I Working Group Meeting, Bethesda, Md., May 29, 1974.

6. Slavik, M.: Updata on Phase I clinical studies sponsored by the Division of Cancer Treatment. Southwest Oncology Group Meeting, Kansas City, June 7, 1974.

7. Penta, J. S.: Federal regulatory requirements for the Use of BCG, C. parvum, and MER BCG in the treatment of human malignancy. Conference on Immunotherapy, National Cancer Institute, Bethesda, Md., July 11, 1974.

8. Penta, J. S.: (a) Mechanism of action of DTIC (NSC 45388) (b) Summary of Phase I clinical trials for ongoing studies, (c) Federal regulatory guidelines and the use of immuno-adjuvant agents. Central Oncology Group, Santa Monica, California, September 4-6, 1974.

10. Slavik, M.: Nucleoside analogues in the treatment of neoplastic and non-neoplastic diseases. New York Academy of Science Conference on the Chemistry, Biology, and Clinical Use of Nucleoside Analogues, New York, September 5, 1974.

11. Slavik, M.: New drugs available for Phase II studies in bronchogenic carcinoma. VA Lung Group Meeting, Spokane, Washington, September 20, 1974.

12. Penta, J. S.: (a) Durg requirements for high-dose methotrexate - citrovorum factor rescue studies, (b) Drug requirements for L-Asparaginase studies, (c) FDA Guidelines and the use of immuno-adjuvants agents for treating human malignancies. Children's CAncer Study Group, New Orleans, Louisiana, October 1, 1974.

13. Penta, J. S.: Summary of the differences in viabil unit counts for Pasteur (low-dose) BCG, Tice BCG, Connaught BCG, and Pasteur (high-dose) BCG. Immunotherapy Subcommittee of the Southwest Oncology Group, Oklahoma City, Oklahoma, October 9, 1974.

14. Slavik, M.: Drug development and experimental design of clinical studies in the Division of Cancer Treatment, NCI. Carola Erba Foundation, Milan, Milan, Italy, October 16, 1974.

15. Carter, S. K. and Slavik, M.: A survey of new compounds clinically investigated. Conference on Advances in Cancer Chemotherapy, XI International Cancer Congress, Florence, Italy, October 22, 1974.

16. Slavik, M. and Carter, S. K.: Quantitative analysis of toxicity of anticancer drugs in man. Workshop on Clinical Chemotherapy, XI International Cancer Congress, Florence, Italy, October 25, 1974.

17. Penta, J. S.: Investigational new anticancer drugs. Department of Surgery, Ohio State University Medical School, Columbus, Ohio, October 30, 1974.

18. Carter, S. K., Slavik, M., and Wasserman, T. H.: Overview of single agent testing of standard drugs and new investigational agents in solid tumors. Conference on Chemotherapy, Houston, Texas, November 8, 1974.

19. Slavik, M.: Pyrazofurin and germanium azaspirane. Overview on clinical results. Decision Network Meeting, Bethesda, Maryland, November 11, 1974.

20. Slavik, M.: New drugs available for Phase II studies. Brain Tumor Study Group Meeting, Bethesda, Maryland, November 14, 1974.

21. Penta, J. S.: Integration of pharmaology into combination cancer chemotherapy. Weekly symposium, St. Mary's Hospital, Grand Rapids, Michigan. December 2, 1974.

22. Slavik, M.: Adriamycin activity in genitourinary and gynecologic malignancy. Five New Drug Seminar on Adriamycin. Washington-Hilton Hotel, December 17, 1974.

23. Slavik, M.: Iphosphamide, Summary of clinical studies in germany. New Drug Liaison Meeting, February 25, 1975.

24. Slavik, M.: Comparative analysis of aureolic acid analogues. 76th Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, March 20, 1975.

25. Slavik, M.: Integration of clinical pharmacology in cancer chemotherapy. Symposium: Cancer Chemotherapy, at the 76th Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, Washington-Hilton Hotel, March 21, 1975.

26. Slavik, M.: Investigational drugs in cancer chemotherapy. International Symposium on Hematology and Clinical Oncology, Dallas, Texas, March 27, 1975.

27. Slavik, M.: New anticancer drugs sponsored for clinical studies by the Division of Cancer Treatment, NCI. National Cancer Congress, Ankara, Turkey, April 4, 1975.

28. Penta, J. S.: Pharmacokinetics and pharmacodynamics of metallic compounds in cancer chemotherapy. East Tennessee Cancer Research Center, Oak Ridge National Laboratories, Knoxville, Tennessee, April 29, 1975.

DIVISION OF CANCER CONTROL AND REHABILITATION

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1974 through June 30, 1975

TABLE OF CONTENTS

Page

DIRECTOR'S REPORT 1

INTERVENTION PROGRAMS 5

Prevention Branch 7

Contract Narratives 9

Detection, Diagnosis and Pretreatment Evaluation Branch 11

Contract Narratives 12

Treatment, Rehabilitation and Continuing Care Branch 63

Contract Narratives 65

SUPPORTIVE SERVICES 113

Evaluation Branch 115

Contract Narrative 116

Resources Branch 117

Contract Narratives 118

Communications Branch 121

Contract Narratives 123

Liaison Branch 127

COMMUNITY ACTIVITIES PROGRAM 129

ANNUAL REPORT

DIVISION OF CANCER CONTROL AND REHABILITATION

July 1, 1974 - June 30, 1975

DIRECTOR'S REPORT

Cancer Control is mandated by the National Cancer Act of 1971 to ensure the rapid translation of existing information and the new leads emerging from research into community practice. Originally organized as a program in the OD-NCI, the National Cancer Institute elevated its cancer control efforts to a divisional status in 1974. Divisional status was approved in September 1974. The importance of Control in the National Cancer Program was reemphasized in mid-1974 with the updating of the National Cancer Plan, which designated cancer control as its second major mission, the first being research.

In the nine months of its existence, the new Division has:

- Continued to develop, implement and evaluate all cancer control and rehabilitation programs of the National Cancer Program to coordinate an assault on the incidence, morbidity and mortality from cancer.
- Reorganized into a Divisional structure allowing that the traditional intervention activities be supported by new functions which make possible an orderly system of identifying, field testing, demonstrating, evaluating and promoting the latest cancer control knowledge.
- Administered funding through both grants and contracts.
- Established a Cancer Control Communications Network in all 17 Comprehensive Cancer Centers which provides every community in the nation with immediate access to up-to-date information on cancer.
- Taken the initiative to fund over 200 grants and contracts in every region of the nation to assure use of cancer control knowledge.
- Initiated a program in rehabilitation research. Except for research in rehabilitation, DCCR does not support research to develop new methods and techniques.
- Begun an industrial program which monitors workers for prevention of the effects of exposure to carcinogens.
- Improved the early detection rate for women with breast cancer.

- Completed Pap tests on approximately 500,000 women, many of whom had never had a test.

Although cancer control is in its third year of operation as a program area of the National Cancer Institute, it is only in the past two years that funding levels have been sufficient enough to permit major program activities, and it is only during this fiscal year that a fully integrated organizational capability has existed. DCCR has committed its allocated FY 75 budget of $50 million.

Despite this brief existence, the Division has received recognition for fulfilling its obligation to get research findings to communities and physicians. This year the Division received the Gerard B. Lambert Award for its sponsorship of professional education and demonstrations through the Cancer Control Treatment project. These programs established for acute childhood leukemia and lymphoma have been sending out specially trained doctors, nurses, and technicians to deliver knowledge to groups of doctors and hospital staff through seminars, lectures, tumor clinics and special consultations. In the past year, cancer experts have served as "circuit riders" bringing information about new treatments and diagnostic procedures to doctors in small towns and cities.

Concern for reaching out into the community was also evident in the accelerated efforts of the Division to establish the new community-based cancer control program, often referred to as the "saturation program." The program involves selected communities in producing a coordinated effort against cancer which includes public medical schools, cancer centers, private physicians, voluntary groups such as the American Cancer Society, and State and local health departments. These communities must have in operation a population-based cancer data system. The long-term mobilization of medical and lay capabilities and resources relies upon the use of new cancer control knowledge by the health community and the motivation of the public to adopt new health practices. Cost-sharing is a requirement of the saturation program, with communities participating on a 50-50 matching basis as their programs become fully operational. Final review of proposals occurred near the end of the fiscal year.

To support communities in their efforts to undertake effective cancer control projects, the Division funded 17 Cancer Control Communications Offices in the Comprehensive Cancer Centers. A primary feature of this program is a WATS system which can be used by anyone anywhere in America to have questions about cancer answered. The Cancer Control Communications programs will enable the Cancer Centers to extend their reach until it meets the needs of all 50 states. The newly installed WATS system to the Cancer Centers will be of particular value to the public and health professionals, who are assured of a quick response to questions they may have about cancer.

Among the nearly 200 cancer control activities funded by the Division, the 27-city breast cancer detection projects jointly funded by DCCR and ACS has produced the most vivid results to date. Through the use of mammography, thermography and clinical examination, 77 per cent of the women found to have cancer were detected in the most favorable stage. This compares favorably with the usual 50 per cent early detection rate.

The Division continued to work closely with other health organizations, especially in screening projects. One such project, conducted with State Health Departments, had provided Pap tests for approximately 500,000 women by the end of the fiscal year. Data for evaluation purposes became available late in the fiscal year and will be analyzed to determine if the program is reaching those at greatest risk.

The Division is now directing control projects which demonstrate how to care for a patient's entire needs for cancer rehabilitation, including psychosocial rehabilitation and the training of professional personnel in rehabilitation techniques. The restoration of a cancer victim to as near normal a state as possible deals not only with the patient but with the institutions and individuals which influence his life. New at-home rehabilitation programs were funded in late fiscal 1975 and a study of employer attitudes toward hiring or reinstating the cancer patient was undertaken as a prelude to changing negative attitudes.

As a basic first step in preventing cancer, the Division encourages an extensive cancer education program. A contract was entered into with the National Clearinghouse for Smoking and Health in an attempt to learn why teenagers smoke and to discover more effective methods of discouraging them from smoking. A series of seminars with eight different industrial groups is exploring ways to minimize exposure to carcinogens.

Several major meetings were sponsored by DCCR during FY 75. DCCR sponsored the meeting, "Cancer Control and the Behavioral Sciences," in San Antonio, Texas, on January 20-22, 1975, which attracted dozens of specialists in oncology, psychiatry, psychology, sociology, etc., to explore behavioral considerations as these relate to cancer control. It was the first such conference convened to explore the state of the art in behavior.

In December 1974, DCCR jointly sponsored with DCCP and ACS the workshop on "Persons at High Risk of Cancer: An Approach to Cancer Etiology and Control" in Key Biscayne, Florida. The workshop examined the state of the art on risk factors for both cancer control and cancer research. Proceedings of this workshop and of the behavioral meetings will be published shortly in monograph form for wide distribution. Additionally, the Third World Conference on Smoking and Health was held in New York City on June 2-5, 1975. This meeting was jointly sponsored by DCCR and ACS.

The Division expanded its inquiry into cancer control techniques to the Soviet Union in 1975. Representatives of the Division participated in meetings in Leningrad in May 1975 to explore the development of mutual exchange programs between the two nations in cancer control.

The reorganization of cancer control and rehabilitation into a Division of the National Cancer Institute provided an opportunity to add new functions which can help stimulate and organize public support for and participation in the intervention programs. The Division is now composed of three main program activities: Supportive Services, Intervention Programs and Community Activities, each with an Associate Director.

Supportive Services provides those activities common to all Divisional activities and include planning and evaluation services, resources, communications and liaison. The Intervention area is concerned with field testing and evaluating all new or existing technologies in limited community settings. The Community Activities takes responsibility for demonstrating proved and practical techniques in large community efforts. Within this organizational framework, a unified approach to a total cancer control program is possible since new channels of communication with a variety of audiences have been opened.

Each of the three main program activities are assisted by a Technical Review Committee, made up of non-government experts who provide scientific peer review for contracts. The grant applications submitted to DCCR are reviewed by the grant study section, CCGRC. The CCRAC continues as the Division's advisory body. To assist CCRAC, two special working groups have been organized. The first, on Reimbursement, is to develop information on payment to aid DCCR contractors and grantees to develop self support at the termination of Federal funding. The second, on Prevention, will advise on new approaches to cancer control prevention.

Governmental liaison activities of DCCR have continued in full swing. Discussions have begun with SRS on Vocational Rehabilitation and rural health. Close relationships with several groups in other HEW health agencies have been initiated and include Health Services Administration, National Center for Health Statistics, and the National Center for Health Services Research of the Health Resources Administration.

These primary activities of FY 1975 are but a part of a national cancer control program which seeks to make certain that technology transfer proceeds in an orderly and rapid fashion. New organizational changes for cancer control and the implementation of new and innovative programs indicate that the emphasis on control in the National Cancer Plan will provide the rapid utilization of the products of cancer research.

INTERVENTION PROGRAMS

The end result of cancer research must be community-level action programs which reach out to those at greatest risk. The Intervention activity field tests new or improved cancer control techniques and sponsors demonstration programs of proven knowledge so models are created which can be replicated throughout the nation.

These responsibilities are met through the efforts of the Prevention; the Detection, Diagnosis and Pretreatment Evaluation; and the Treatment, Rehabilitation and Continuing Care Branches of the Division. The programs of each of these branches provide the experiential base for all other activities of the Division.

The Intervention activities are concerned with cancer control and rehabilitation from several perspectives, beginning with an examination of conditions which could cause cancer, to the continuing care of cancer patients. Included in the purview of Intervention are massive screening programs, such as the cervical cytology programs conducted in cooperation with State Health Departments, environmental surveillance programs (such as that being conducted with asbestos workers in Tyler, Texas), to an examination of the psychosocial obstacles to restoration of the cancer patient to full membership in society.

Intervention activities explore methods for cancer control which, if evaluated as effective, provide a basis for continuation without Federal involvement. In this way the Intervention programs not only provide direct services to those at risk but perform the equally valuable service of providing model systems which can be implemented with confidence.

As information is generated by the Intervention programs, it is made available to other segments of the Division for dissemination beyond the limits of the field test. This helps assure that the rest of the nation has the same knowledge of new or improved methods of cancer control.

PREVENTION BRANCH

The objective of the DCCR Prevention Branch is to assure that practical and effective methods and techniques of reducing exposure to carcinogenic agents are available to and utilized by the public and health professionals. This involves identification and evaluation of ways of reducing both exposure to and the presence of carcinogenic agents, the substitution and modification of agents and prevention education.

During FY 75, the Branch funded the educational efforts of the National Clearinghouse for Smoking and Health in Atlanta, which included studies of teenage cigarette smoking in the U. S. The Branch also co-sponsored, with the American Cancer Society, the Third World Conference on Smoking and Health.

A survey of cancer control laws in the U. S. and a study to identify high-risk occupational groups were begun. A demonstration cervical cytology program in the Southwest involved both a preventive health information program and screening of Indian women at high risk to cervical and uterine cancer. The continuation of this program will stress secondary prevention of precancerous conditions.

Another demonstration initiated among asbestos workers in Tyler, Texas, sought to provide health education to both the workers and their families, to conduct medical surveillance for detection of precancerous lesions and early lung cancer and to provide appropriate treatment.

Continued medical surveillance of selected target populations significantly exposed to designated carcinogens will be a program emphasis during the coming year. The purpose of the surveillance is to set up models for monitoring populations exposed to carcinogens and for identifying conditions which may provide early warnings of malignancy. The Tyler, Texas, program on asbestos workers will be continued. A new program for workers exposed to vinyl chloride in Louisville, Kentucky, was initiated in late FY 75. Development of medical surveillance programs for other high-risk populations will be a high priority for FY 76.

A carcinogen control program will be a feature of the work of the Branch. Key carcinogens warranting control activity will be identified and prioritized, and practical control and prevention strategies will be developed.

Workshops, seminars and conferences for reviewing, evaluating and disseminating cancer prevention information are being scheduled for FY 76. An industrial stewardship program relating to cancer risks from chemical manufacture was initiated during the last quarter of FY 75 and will be carried forward during the next two fiscal years. Eight seminars will be conducted with various industries to provide an understanding of the effect of occupational exposure to carcinogens. Preventions will be identified and intervention preventions developed. Industries covered will include agriculture, chemical, petroleum, plastics, rubber, mining and metals.

Because a number of tumors in this country are believed to be environmentally conditioned and are preventable, the Prevention Branch will conside grant proposals related to alerting health professionals to cancer risk-reducing measures and those concerning motivation of people to take steps to reduce cancer risk. Included will be proposals for identification of high- and low-risk groups, the development of models for prevention and intervention activities and incorporation of prevention strategies into health maintenance and the health care system.

Six radiation physics reference centers are supported by DCCR to monito the diagnostic and therapeutic radiological aspects of all DCCR contracts involving this modality.

CONTRACT SUMMARY

Title: Survey of Cancer Control Laws

Principal Investigator: G. Seifert; J. Benyan
11600 Nebel Street
Rockville, Maryland 20852

Performing Organization: Health Law Center, Aspen Systems Corporation

Contract Number: 45117

Starting Date: 06/01/74 Expiration Date: 01/31/76

Objective: Collect, review, catalog and analyze present legislation (Federal, state and local) pertaining to cancer control activities in the areas of cause and prevention, detection, treatment, rehabilitation, education and training. Prepare for continuing survey of newly enacted legislation or proposed and significant actions based on legislation.

Proposed Course: Aspen presented to DCCR how they would survey, interpret, and catalog laws. This was phase one which was completed before phase two which is survey.

Progress: Phase one and phase two completed. Survey and cataloging of laws complete. Verifying still in progress.

Significance for Cancer Control Program: To identify preventive methods in multiple sites.

Project Officer: Dr. Woolridge

FY 75 Funds: $19,742

CONTRACT SUMMARY

Title: Agreement Study of Carcinogenic Effects of Smoking

Principal Investigator: Horn
CDC, 1600 Clifton Road
Bethesda, Maryland 20016

Performing Organization: National Clearinghouse for Smoking and Health

Contract Number: 40001

Starting Date: 07/01/73 Expiration Date: 06/30/74

Objective: The Center will maintain, evaluate and report on programs, contracts, purchase orders and other appropriate mechanisms to: (1) develop smoking education activities carried on by professional health workers; (2) continue to assess San Diego community organization project; (3) carry on and evaluate the techniques developed for smoking education in schools; (4) evaluate public education programs; (5) develop a systematic documentation of accomplishments.

Proposed Course: This is second year of two-year agreement program continued from first year. An analysis agreed upon and started.

Progress: Teenage survey of smoking completed, analyzed, and written. Adult survey started. Plans for professional survey agreed upon. Merit review of program planned.

Significance for Cancer Control Program: To identify, demonstrate, and promote preventive methods in the lung and urinary tract.

Project Officer: Dr. Woolridge

FY 75 Funds: $753,900

DETECTION, DIAGNOSIS AND PRETREATMENT EVALUATION BRANCH

The two main objectives of the Detection, Diagnosis and Pretreatment Evaluation Branch are: (1) to assure, with the aid of health professionals and other groups, continuous assessment of current practices and development of principles for the optimal detection, diagnosis and pretreatment evaluation of cancer patients; and (2) to assure that practical and effective cancer screening/detection methods and optimal methods and techniques for diagnosis and pretreatment evaluation are avilable to and utilized by persons with precancerous and cancerous lesions.

In cooperation with the Division of Cancer Biology and Diagnosis and the American Cancer Society, the DDPEB is supporting 27 breast cancer detection programs designed to evaluate the value of patient history, clinical exams, thermography and mammography (film and xeroradiography) as early detection methods. Other cooperative efforts with the Division of Cancer Biology and Diagnosis include controlled studies to evaluate the efficacy of thermography and projects to detect lung cancer by sputum cytology and fiberoptic bronchoscopic examination.

In cooperation with State and Territorial Health Departments, the DDPEB is sponsoring 36 cervical cancer screening programs. Prime objectives are to reach high-risk populations who have not availed themselves of preventive health care in the past and to critically evaluate the program in order to define aspects which are practical, effective and acceptable to both consumers and providers. The ultimate goal is to define and demonstrate how effective screening/detection/diagnosis strategies can be continued by the local community of health providers. The Branch is also involved in other efforts to assess and evaluate the frequency of screening necessary to effect control of cervical and uterine cancer.

In order to demonstrate the proper utilization of interdisciplinary approaches toward the early detection, diagnosis and thorough pretreatment evaluation of head and neck cancer, the DDPEB is now supporting nine prototype demonstration projects for head and neck cancer. Another project involves field testing and evaluation of emerging concepts and techniques as applied to the early identification and pretreatment evaluation of persons with cutaneous malignant melanoma.

The DDPEB is promoting education, training methods and techniques to improve the health professional's understanding and use of optimal diagnostic regimens. Seven such projects targeted toward the practicing (community) radiologists and radiologic technicians involve mammography training for the early detection of breast cancer. Other on-going projects involve the training of thermography technologists and planning for pathology reference centers.

CONTRACT SUMMARY

Title: Thermography Tech Training Program

Principal Investigator: Dr. Haberman
Health Sciences Center
Oklahoma City, Oklahoma

Performing Organization: University of Oklahoma

Contract Number: 55163

Starting Date: 09/09/74 Expiration Date: 09/08/75

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $46,000

CONTRACT SUMMARY

Title: Prototype Comparative Network Demonstration Project for Head and Neck Cancer

Principal Investigator: Dr. Hendrickson
1753 W. Congress Parkway
Chicago, Illinois 60612

Performing Organization: Rush-Presbyterian-St. Luke's Center

Contract Number: 45159

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish network of cooperating hospitals to develop and implement cancer control activities relating to diagnosis, treatment and rehabilitation of all stages of head and neck cancer. Contractor serves as major cancer hospital to which network will be linked. Serves as a demonstration project to the medical community for a multidisciplinary approach to total patient care. This contract performed in conjunction with Illinois Cancer Center and Northwestern University.

Significance for Cancer Control Program: To identify, field test, and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods of the head and neck.

Project Officer: Drs. Hamner and Choksi

FY 75 Funds: $8,994

CONTRACT SUMMARY

Title: Prototype Comparative Network Demonstration Project for Head and Neck Cancer

Principal Investigator: Dr. Lore
P. O. Box 7126
Albany, New York 12224

Performing Organization: Research Foundation of State University of New York

Contract Number: 45154

Starting Date: 06/30/74 Expiration Date: 06/29/77

Objective: To establish network of cooperating hospitals to develop and implement cancer control activities relating to diagnosis, staging, treatment and rehabilitation of all stages of head and neck cancer. Contractor serves as major cancer hospital to which network will be linked. Serves as demonstration program to medical community for multidisciplinary approach to total patient care. 200 patients/contract. This contract performed in conjunction with the New York State Department of Health, Roswell Park, Buffalo, New York.

Significance for Cancer Control Program: To identify, field test and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods of the head and neck.

Project Officer: Drs. Hamner and Choksi

FY 75 Funds: $295,000

CONTRACT SUMMARY

Title: Prototype Comparative Network Demonstration Project for Head and Neck

Principal Investigator: Dr. Sisson
633 N. Clark Street, Evanston
Chicago, Illinois 60601

Performing Organization: Northwestern University

Contract Number: 45132

Starting Date: 06/07/74 Expiration Date: 06/06/77

Objective: Establish network of cooperating hospitals to develop and implement cancer control activities relating to diagnosis, staging, treatment and rehabilitation of all stages of head and neck cancer. Contractor serves as major cancer hospital to which network will be linked. Serves as demonstration program to medical community for multidisciplinary approach to patient care. Participants are Rush-Presbyterian-St. Luke's Medical Center to establish single network under jurisdiction of the Illinois Cancer Center. 200 patients/contract.

Significance for Cancer Control Program: To identify, field test and evaluate diagnostic, pretreatment evaluation, treatment, follow-up, continuous care methods and rehabilitative methods in the head and neck.

Project Officer: Drs. Hamner and Choksi

FY 75 Funds: $212,000

CONTRACT SUMMARY

Title: Prototype Comparative Network Demonstration Project for Head and Neck Cancer

Principal Investigator: James H. Erandenburg
750 University Avenue
Madison, Wisconsin 53706

Performing Organization: Board of Regents, University of Wisconsin System

Contract Number: 45130

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Establish network of cooperating hospitals to develop and implement cancer control activities relating to diagnosis, staging and treatment and rehabilitation of all stages of head and neck cancer. Contractor serves as major cancer hospital to which network will be linked. Serves as demonstration program to medical community for multidisciplinary approach to patient care. 200 patients/contract.

Significance for Cancer Control Program: To identify, field test and evaluate diagnostic, pretreatment evaluation, treatment, follow-up, continuous care methods and rehabilitative methods in the head and neck.

Project Officer: Drs. Hamner and Choksi

FY 75 Funds: $198,000

CONTRACT SUMMARY

Title: Cytological Demonstration Project for SW American Indian Population

Principal Investigator: Dr. Scott W. Jordan
Albuquerque, New Mexico 87106

Performing Organization: University of New Mexico School of Medicine

Contract Number: 45071

Starting Date: 03/15/74 Expiration Date: 03/14/77

Objective: Implementation of a cervical cancer detection project to provide diagnostic cytology and treatment program and education related to cancer of the uterine cervix for the Navajo Indians and a defined population of medically indigent non-Indians. The function of the proposed project in the area of treatment would be to ensure that patients have been contacted by the Indian Health Service to arrange for treatment, receive treatment, and collect treatment and follow-up data.

Proposed Course: Letter of agreement with Indian tribe headquarters reached. Training of Indian health medics undertaken. Health education of tribe agreed upon before starting.

Progress: Completed 25,205 PAP smears and examination first year. First comparative study of Indian PAP results to SEER Base done. Education program underway.

Significance for Cancer Control Program: To identify preventive methods in the female genitals.

Project Officer: Dr. Woolridge

FY 75 Funds: $82,744

CONTRACT SUMMARY

Title: State Cervical Cancer Screening Program

Principal Investigator: Dr. Greenwald
84 Holland Avenue
Albany, New York

Performing Organization: New York State Department of Health and Health Research, Inc.

Contract Number: 45091

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Expand existing cervical cancer screening programs with emphasis on low income or indigent women. Includes definite diagnostic procedures where pap test is positive or suspicious. Provides for rescreening every twelve months for duration of contract. Anticipate approximately 219,600 screenings over contract period of three years. 67,200 screening reports 12 months after DOC; 72,600 screening reports 24 months after DOC; 79,800 screening reports 36 months after DOC.

Significance for Cancer Control Program: To evaluate, demonstrate and promote screening and detection techniques in the female genitals.

Project Officer: Dr. McQueen

FY 75 Funds: $808,805

CONTRACT SUMMARY

Title: Lung Cancer Control Detection and Therapy Phase II

Principal Investigator: Dr. John K. Frost
Charles and 34th Streets
Baltimore, Maryland 21218

Performing Organization: The Johns Hopkins University

Contract Number: 45037

Starting Date: 09/01/73 Expiration Date: 07/15/78

Objective: To screen individuals who are at high risk of developing lung cancer and who offer the best possibility for evaluating: (1) the efficacy of sputum cytopathology in detecting pulmonary neoplasms at an early stage; (2) number of cases missed by screening procedures; (3) efficacy of techniques for prompt localization of early lesions before progression to x-ray; (4) survival rates of screened vs. unscreened; (5) efficiency in screening in reducing mortality.

Significance for Cancer Control Program: To field test screening and detection techniques in the lung.

Project Officer: Drs. Greenberg and Hamner

FY 75 Funds: $407,941

CONTRACT SUMMARY

Title: Effect of Early Detection and Treatment on Survival From Lung Cancer

Principal Investigator: Dr. Myron D. Melamed
44 East 68th Street
New York, New York 10021

Performing Organization: Memorial Hospital for Cancer and Allied Disease

Contract Number: 45007

Starting Date: 09/01/73 Expiration Date: 07/31/78

Objective: To evaluate efficacy of sputum cytology in early detection of lung cancer in population of 10000 male heavy smokers (age 45-69). Half of population receive only x-ray screening, others receive cytopathologica exam. Fiberoptic bronchoscopy is used to locate tumors. All neoplasms to be surgically treated. Minimum 5 year follow-up.

Significance for Cancer Control Program: To field test screening and detection techniques in the lung.

Project Officer: Drs. Greenberg and Hamner

FY 75 Funds: $550,604

CONTRACT SUMMARY

Title: Study of the Incidence and Natural History of Genital Tract Anomalies and Cancer in Offsprings Exposed in Utero to Synthetic Estrogens

Principal Investigator: Dr. Kaufman
1200 Moursund Avenue
Houston, Texas 77025

Performing Organization: Baylor College of Medicine

Contract Number: 45092

Starting Date: 06/24/74 Expiration Date: 06/23/77

Objective: Identify and study offspring of women who received synthetic estrogens during pregnancy between 1943 and 1963. Repeat examinations at regular intervals, perform diagnosis and follow-up and treatment as necessary. Approximately 1,000 offspring to be examined on this contract.

Proposed Course: The contractor will establish standard definition of eligibles and develop suitable approaches to elicit eligibles through information campaigns to physicians and general public via mass media. Evaluation will be performed and reported.

Progress: 153 patients have been examined. Chart reviews of physicians in Houston area are being continued. Contact has been made with health services at local universities and consent forms have been sent.

Project Officer: Dr. Hamner

FY 75 Funds: $112,810

CONTRACT SUMMARY

Title: Study of the Incidence and Natural History of Genital Tract Anomalies and Cancer in Offsprings Exposed in Utero to Synthetic Estrogens

Principal Investigator: Townsend
University Park
Los Angeles, California 90007

Performing Organization: University of Southern California

Contract Number: 45122

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Identify and study offspring of women who received synthetic estrogens during pregnancy between 1943 and 1963. Repeat examinations at regular intervals, perform diagnosis and follow-up and treatment as necessary. Approximately 1,000 offspring to be examined on this contract.

Significance for Cancer Control Program: To field test diagnostic and pretreatment evaluation in the female genitals.

Project Officer: Dr. Hamner

FY 75 Funds: $196,000

CONTRACT SUMMARY

Title: Study of the Incidence and Natural History of Genital Tract Anomalies and Cancer in Offsprings Exposed in Utero to Synthetic Estrogens

Principal Investigator: Dr. Herbst
Boston, Massachusetts 02114

Performing Organization: Massachusetts General Hospital

Contract Number: 45157

Starting Date: 06/27/74 Expiration Date: 06/26/77

Objective: To identify and study offspring of women who received synthetic estrogens during pregnancy between 1943 and 1963. Repeat examinations at regular intervals, perform diagnosis, follow-up and treatment as necessary. Approximately 1,000 offspring to be examined on this contract.

Proposed Course: The contractor will establish standard definition of eligibles and will develop suitable approaches to elicit eligibles through information campaigns to physicians and general public via mass media. Evaluation will be performed and reported.

Progress: Have hired personnel; purchased necessary equipment; pretested 11 individuals and findings have been evaluated.

Significance for Cancer Control Program: To field test diagnosis and pretreatment evaluation in the female genitals.

Project Officer: Dr. Hamner

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Study of the Incidence and Natural History of Genital Tract Anomalies and Cancer in Offsprings Exposed in Utero to Synthetic Estrogens

Principal Investigator: Drs. Decker and Kurland
200 First Street, SW
Rochester, Minnesota 55901

Performing Organization: Mayo Foundation

Contract Number: 45124

Starting Date: 06/01/74 Expiration Date: 06/30/77

Objective: Identify and study offspring of women who received synthetic estrogens during pregnancy between 1943 and 1963. Repeat examinations at regular intervals, perform diagnosis and follow-up and treatment as necessary. Develop manual of operations governing procedures at all centers and conduct training in performance of these procedures. Coordinate with three other contractors performing similar studies.

Significance for Cancer Control Program: To field test diagnostic and pretreatment evaluation in the female genitals.

Project Officer: Dr. Hamner

FY 75 Funds: 0

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. William A. Maddox
University Station
Birmingham, Alabama 35294

Performing Organization: Board of Trustees, University of Alabama

Contract Number: 45129

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test and evaluate diagnostic, pretreatment evaluation, treatment, follow-up, continuous care methods and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $215,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Whitney
P. O. Box 1668
Wilmington, Delaware 28999

Performing Organization: Wilmington Medical Center, Executive Offices

Contract Number: 45151

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test, and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $260,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Moore
University of Louisville
Louisville, Kentucky 40201

Performing Organization: University of Louisville Foundation, Inc.

Contract Number: 45149

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients. Subcontractors include Trover Clinic, Jewish Hospital and Norton Children's Hospital.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $215,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Foster
85 South Prospect Street
Burlington, Vermont 05401

Performing Organization: University of Vermont School of Medicine

Contract Number: 45145

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients. Contract being performed in conjunction with Dartmouth College.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test, and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $470,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Simeon Cantril
P. O. Box 7999
San Francisco, California 94120

Performing Organization: West Coast Cancer Foundation Pacific Medical Center

Contract Number: 45143

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test, and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $350,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. R. W. Sponzo
47 New Scotland Avenue
Albany, New York 12208

Performing Organization: Albany Medical College of Union University

Contract Number: 45140

Starting Date: 06/26/74 Expiration Date: 06/25/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $270,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Anne Carter
P. O. Box 7126, Albany
Brooklyn, New York

Performing Organization: Research Foundation of State University of New York

Contract Number: 45139

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test, and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $400,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Arthur Hoge
825 NE 13th Street
Oklahoma City, Oklahoma 73104

Performing Organization: Oklahoma Medical Research Foundation

Contract Number: 45137

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of cancer patients. Work to be performed in conjunction with the University of Oklahoma Health Sciences Center.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To identify, field test and evaluate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods of the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $340,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Huguley
718 Woodruff Building
Atlanta, Georgia 30322

Performing Organization: Georgia Cancer Management Network, Emory University

Contract Number: 55131

Starting Date: 09/15/74 Expiration Date: 09/14/77

Objective: To establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Significance for Cancer Control Program: To field test, evaluate and demonstrate diagnosis and pretreatment evaluation, treatment and follow-up care methods, and rehabilitative methods of the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $149,742

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Paul Engstrom
7701 Burholme Avenue
Philadelphia, Pennsylvania 19111

Performing Organization: The Institute for Cancer Research

Contract Number: 45055

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Establish networks of community hospitals as demonstrations.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches will be explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Project Officer: Dr. Hamner

FY 75 Funds: $265,000

CONTRACT SUMMARY

Title: Prototype Network Demonstration Project in Breast Cancer

Principal Investigator: Dr. Marchant
171 Harrison Avenue
Boston, Massachusetts 02111

Performing Organization: New England Medical Center Hospital

Contract Number: 45054

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Establish networks of community hospitals as demonstration programs to the medical community for the utility and need of an organized and multidisciplinary approach to the total care of breast cancer patients.

Proposed Course: The contractor will develop uniform approaches to diagnosis and staging and evaluate uniform treatment protocols based on diagnosis and staging criteria; several approaches have been explored; evaluation is envisioned.

Progress: Two meetings have been held with contractors; implementation plans have been submitted. A third meeting is scheduled for June. Renegotiation of cost proposal is ongoing.

Project Officer: Dr. Hammer

FY 75 Funds: $175,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Leslie W. Whitney
P. O. Box 1668
Wilmington, Delaware 19899

Performing Organization: Wilmington Medical Center

Contract Number: 45047

Starting Date: 01/01/74 Expiration Date: 12/31/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $185,327

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Fred I. Gilbert, Jr.
Hotel and Bishop Streets
Honolulu, Hawaii 96813

Performing Organization: Pacfic Health Research Institute

Contract Number: 45046

Starting Date: 12/19/73 Expiration Date: 12/18/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $171,966

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Ward
151 East Bannock
Boise, Idaho 83702

Performing Organization: Mountain States Tumor Institute

Contract Number: 35029

Starting Date: 06/25/73 Expiration Date: 06/24/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Thomas Carlile
1000 Seneca Street
Seattle, Washington 98101

Performing Organization: Virginia Mason Research Center

Contract Number: 35028

Starting Date: 06/15/73 Expiration Date: 06/14/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $200,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. R. Boudet
Rainbow Blvd. at 39th Street
Kansas City, Kansas 66103

Performing Organization: University of Kansas Medical Center

Contract Number: 35026

Starting Date: 06/19/73 Expiration Date: 06/18/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hammer

FY 75 Funds: $183,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Myron Moskowitz
Eden and Bethesda Avenues
Cincinnati, Ohio 45229

Performing Organization: University of Cincinnati Medical Center

Contract Number: 35024

Starting Date: 06/28/73 Expiration Date: 06/26/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officers: Drs. Pomerance and Hamner

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Marvin V. McClow
Barrs Street and St. Johns Avenue
Jacksonville, Florida 32204

Performing Organization: St. Vincent's Medical Center

Contract Number: 55210

Starting Date: 01/27/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Project Officer: Dr. Hamner

FY 75 Funds: $177,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Joann Haberman
P. O. Box 26901
Oklahoma City, Oklahoma 73190

Performing Organization: University of Oklahoma Health Sciences Center

Contract Number: 35020

Starting Date: 06/18/73 Expiration Date: 06/17/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hammer

FY 75 Funds: $200,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Brown
204 Woodruff Building
Atlanta, Georgia 30322

Performing Organization: Emory University School of Medicine

Contract Number: 35016

Starting Date: 05/09/73 Expiration Date: 05/08/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreens).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $180,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Condict Moore
Health Science Center
Louisville, Kentucky 40201

Performing Organization: University of Louisville School of Medicine

Contract Number: 35012

Starting Date: 04/17/73 Expiration Date: 04/16/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Benjamin Rush
100 Bergen Street
Newark, New Jersey 07103

Performing Organization: College of Medicine & Dentistry of New Jersey

Contract Number: 35006

Starting Date: 06/28/73 Expiration Date: 11/01/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen). 3 month time extension, no dollars.

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $183,333

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Philip Strax
200 Madison Avenue
New York, New York 10028

Performing Organization: Guttmann Breast Diagnostic Institute Inc.

Contract Number: 35004

Starting Date: 04/18/73 Expiration Date: 04/17/75

Objective: Co-sponsored with ACS-screens 10,000 new patients annually, ages 35-75. Year 1=10,000; Year 2=18,000 (10,000 new, 8,000 rescreen).

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $340,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. H. P. Constantine
593 Eddy Street
Providence, Rhode Island 02902

Performing Organization: Rhode Island Hospital

Contract Number: 45096

Starting Date: 05/28/74 Expiration Date: 05/27/75

Objective: ACS/NCI cooperative project. Contractor to screen 5,000 women annually in age group 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $250,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Moore
1919 Iaeranch
Houston, Texas

Performing Organization: St. Joseph Hospital

Contract Number: 55100

Starting Date: 10/01/74 Expiration Date: 09/30/75

Project Officer: Drs. Pomerance and Ha[illegible]ner

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. W. Donegan
Business Loop 70 and Garth
Columbia, Missouri 65201

Performing Organization: Cancer Research Center

Contract Number: 45095

Starting Date: 06/01/74 Expiration Date: 05/31/75

Objective: ACS/NCI cooperative project. Contractor to screen 5,000 women annually in age group 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hammer

FY 75 Funds: $250,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. M. J. Goodman
1015 NW 22nd Avenue
Portland, Oregon 97210

Performing Organization: Good Samaritan Hospital

Contract Number: 45088

Starting Date: 06/01/74 Expiration Date: 05/31/75

Objective: ACS/NCI cooperative project. Contractor to screen 5,000 women annually in age group 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $250,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Robert Schweitzer
Hawthorne and Webster Streets
Oakland, California 94609

Performing Organization: Samuel Merritt Hospital

Contract Number: 45068

Starting Date: 02/25/74 Expiration Date: 03/24/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $183,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Donald C. Young
716 Darnell Avenue
Des Moines, Iowa 50316

Performing Organization: Iowa Lutheran Hospital

Contract Number: 45067

Starting Date: 02/27/74 Expiration Date: 06/26/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hammer

FY 75 Funds: $173,080

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Bernard Fisher
3550 Terrace Street
Pittsburgh, Pennsylvania 15213

Performing Organization: University of Pittsburgh School of Medicine

Contract Number: 45065

Starting Date: 02/15/74 Expiration Date: 06/14/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $173,133

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Richard G. Lester
Durham, North Carolina 27710

Performing Organization: Duke University Medical Center

Contract Number: 45064

Starting Date: 02/15/74 Expiration Date: 02/14/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $176,667

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Margaret Abernathy
37th and O Streets, NW
Washington, D. C. 20007

Performing Organization: Georgetown University Medical School

Contract Number: 45062

Starting Date: 02/07/74 Expiration Date: 04/06/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $183,188

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Isard
York and Tabor Roads
Philadelphia, Pennsylvania 19141

Performing Organization: A. Einstein Medical Center and Temple University Hospital

Contract Number: 45058

Starting Date: 01/02/74 Expiration Date: 01/01/76

Objective: ACS/NCI cooperative project. Subcontract to Temple University. Contractor to screen 5,000 women in age group 35-75 within twelve months = Year 1; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $180,737

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. Walter W. Whitehouse
Research Administration Building, North Campus
Ann Arbor, Michigan 48104

Performing Organization: University of Michigan, Division of Research Development Administration

Contract Number: 45049

Starting Date: 03/18/74 Expiration Date: 06/17/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hammer

FY 75 Funds: $185,000

CONTRACT SUMMARY

Title: Breast Cancer Detection Demonstration Project

Principal Investigator: Dr. James E. Youker
561 North 15th Street
Milwaukee, Wisconsin 53233

Performing Organization: Medical College of Wisconsin

Contract Number: 35018

Starting Date: 06/18/73 Expiration Date: 06/17/75

Objective: Co-sponsored with ACS-screens 5,000 new patients annually, ages 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Dr. Hamner

FY 75 Funds: $180,000

CONTRACT SUMMARY

Title: Demonstration Project on Early Detection of Breast Cancer

Principal Investigator: Dr. L. Guiss
University Park
Los Angeles, California 90007

Performing Organization: University of Southern California

Contract Number: 45098

Starting Date: 06/25/74 Expiration Date: 06/24/75

Objective: ACS/NCI cooperative project. Contractor to screen 5,000 women annually in age group 35-75. Year 1=5,000; Year 2=10,000 (5,000 new, 5,000 rescreen).

Significance for Cancer Control Program: To demonstrate screening and detection techniques in the female breast.

Project Officer: Drs. Pomerance and Hamner

FY 75 Funds: $250,000

CONTRACT SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: Dr. Raymond Fink
652 Madison Avenue
New York, New York 10022

Performing Organization: Health Insurance Plan of Greater New York

Contract Number: 35021

Starting Date: 06/28/73 Expiration Date: 09/29/75

Objective: Establishes 3 screening centers for collecting risk group data (ages 45-64) and evaluating efficacy of mammography, thermography and clinical exams as early detection methods. All positives Year 1=7,000 females; Year 2=10,000 females. Pathological diagnosis and therapy.

Significance for Cancer Control Program: To field test screening and detection techniques in the female breast.

Project Officer: Drs. Brodie and Geller

FY '75 Funds: $701,352

CONTRACT SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: J.D. Wallace
1025 Walnut Street
Philadelphia, Pennsylvania 19107

Performing Organization: Thomas Jefferson University

Contract Number: 35027

Starting Date: 06/28/73 Expiration Date: 07/29/75

Objective: Evaluates thermography, mammography and clinical exams as early breast cancer detection modalities. Population of 13,000. Year 1=5,000 females; Year 2=10,000 females.

Significance for Cancer Control Program: To field test screening and detection techniques in the female breast.

Project Officer: Drs. Brodie and Geller

FY 75 Funds: $793,802

TREATMENT, REHABILITATION AND CONTINUING CARE BRANCH

The objective of the Treatment, Rehabilitation and Continuing Care Branch is to insure that optimal treatment and follow-up care methods and techniques are available to and utilized by cancer patients. Utilizing the ever-expanding knowledge derived from biomedical research projects sponsored by the Branch benefits not only cancer patients but also the health professionals who care for them.

Of particular importance in FY 75 were treatment activities demonstrating recently developed methods of chemotherapy and radiation therapy in acute childhood leukemia and Hodgkin's disease in community hospitals. Proper diagnosis and pretreatment evaluation were also objectives of these projects.

The Branch initiated the development of a network of community hospitals for diagnosis, treatment, and rehabilitation of breast cancer, involving 12 primary contractors.

At present, in cooperation with the Division of Cancer Cause and Prevention, the Treatment Branch is undertaking a study in which current diagnosis and treatment practices of prototype cancer diseases in specific geographic areas will be analyzed.

Under a grant is an assessment of current radiation therapy practices in the U. S.

All of these programs aim to develop the means to cure as many patients as possible and to maintain maximum control of the cancerous process in uncured patients.

Rehabilitation plans look to training of cancer rehabilitation teams and development of more functional breast and other protheses.

Thirty-nine projects in nine different areas were initiated in FY 75, the first year of the DCCR rehabilitation and continuing care program. In general, the program and its projects seek to ensure a national capacity to restore cancer patients to as nearly a normal state as possible. Rehabilitation is considered a structured process for restoring maximal physical, psychosocial and vocational functions lost as a result of injury or disease and one that should be initiated after diagnosis, coordinated with the primary treatment planned and continued through and beyond the treatment period.

Program activity stressed developments of comprehensive services such as vocational rehabilitation, physical restoration and supportive techniques and included development of new and improved prosthetic and orthotic devices.

Currently the program funds the training of more than 500 physical therapists, maxillofacial prosthodontists and maxillofacial dental technicians dealing with head and neck cancer. It also supports projects seeking to modify the social and vocational environment for work-able cancer patients.

Future treatment programs of the Branch will focus on pediatric oncology, including the emotional and social adjustment of children with malignancies. Baseline data will be acquired on cost effectiveness of cancer treatment, distribution of treatment to the patient and information on how cancer is managed by a variety of treatment centers. Future plans look to training of cancer rehabilitation teams and development of more functional breast and other prostheses.

CONTRACT SUMMARY

Title: Training Program for Maxillofacial Prosthodontists/ Dental Technician

Principal Investigator: Joe B. Drane
6723 Bertner Avenue
Houston, Texas 77025

Performing Organization: University of Texas System Cancer Center

Contract Number: 45114

Starting Date: 06/28/74 Expiration Date: 06/27/76

Objective: Provide training for prosthodontists in the use of maxillofacial protheses and for training of maxillofacial dental technicians in fabrication of prosthetic devices for rehabilitation of cancer patients with head and neck cancer. Trainees to be heavily involved in patient care, and training includes workshops, laboratory, audio-visual and simulation aids. Technicians to be trained in the construction of intra- and extra-oral and implant prostheses.

Proposed Course: Selection has been done for 1975-76 residency and dental technician programs.

Progress: Curriculum prepared for technicians. Three maxillofacial dental technicians will complete their training.

Significance for Cancer Control Program: To demonstrate diagnostic and pretreatment evaluation in the head and neck.

Project Officer: Dr. Choksi

FY 75 Funds: $146,037

CONTRACT SUMMARY

Title: Training Program for Maxillofacial Prosthodontist/Dental Technician

Principal Investigator: Kenneth Adisman
421 First Street
New York, New York 10010

Performing Organization: New York University Brookdale Dental Clinic

Contract Number: 45113

Starting Date: 06/28/74 Expiration Date: 06/27/78

Objective: Develop and implement training programs for two maxillofacial prosthodontists and two maxillofacial dental technicians per year in use of prostheses for rehabilitation of patients with head and neck cancer and in fabrication of prosthetic devices. Trainees to be heavily involved in patient care, and training to include workshops, laboratory, audio-visual and simulation aids, and in interdisciplinary approach to diagnosis, therapy, follow-up and maintenance.

Proposed Course: 1975-76 candidates for maxillofacial residency and technicians have been selected and four of them will begin training July 1975.

Progress: One maxillofacial prosthodontist and one maxillofacial dental technician will complete their training in November 1975.

Project Officer: Dr. Choksi

FY 75 Funds: $172,135

CONTRACT SUMMARY

Title: Training Program for Maxillofacial Prosthodontist/Dental Technician

Principal Investigator: Norman G. Schaaf
666 Elm Street
Buffalo, New York 14203

Performing Organization: New York State Department of Health

Contract Number: 45101

Starting Date: 06/28/74 Expiration Date: 06/27/76

Objective: Provide training for prosthodontists in the use of maxillofacial prostheses and for training of maxillofacial dental technicians in fabrication of prosthetic devices for rehabilitation of patients with head and neck cancers. Trainees to be heavily involved in patient care, and training to include workshops, laboratory, audio-visual and simulation aids. Technicians to be trained in the construction of intra- and extra-oral and implant prostheses. Program to train two prosthodontists and two dental technicians per year.

Proposed Course: 1975-76 residency and training program selections have been done.

Progress: Two maxillofacial prosthodontists and two maxillofacial dental technicians will complete their training (1974-75).

Project Officer: Dr. Choksi

FY 75 Funds: $139,573

CONTRACT SUMMARY

Title: Training Program for Maxillofacial Prosthodontists/Dental Technicians

Principal Investigator: Lepley
444 East 68th Street
New York, New York 10021

Performing Organization: Memorial Hospital for Cancer and Allied Disease

Contract Number: 45048

Starting Date: 12/01/73 Expiration Date: 11/30/75

Objective: Provides for the development of comprehensive programs of training for maxillofacial prosthodontists and laboratory technicians in the specialized techniques and practices used in the functional and cosmetic rehabilitation of patients with cancer of the head and neck. It provides for the training of an initial class of students under these programs.

Proposed Course: Request to expand the scope of work was granted with an additional 7 months. Hence, now two maxillofacial prosthodontists and two maxillofacial dental technicians will be trained - 1975-76.

Progress: One maxillofacial prosthodontist and two dental technicians will complete their training.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in the head and neck.

Project Officer: Dr. Choksi

FY 75 Funds: $46,482

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. Richard Silver
525 East 68th Street
New York, New York 10021

Performing Organization: Cornell University Medical College

Contract Number: 35025

Starting Date: 06/18/73 Expiration Date: 06/17/76

Objective: Extend progress in chemotherapy and combined modality therapy utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults in hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors.

Proposed Course: The contractor will develop a prototype clinical chemotherapy program by recruitment of previously trained clinical chemotherapy fellows and by utilization of a system already established.

Progress: Case accrual is continuing; patients are being added to the system. Educational program is in operation and functioning well.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continuous care methods for leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $168,143

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. Charlene Holton
1056 East 19th Avenue
Denver, Colorado 80318

Performing Organization: Children's Hospital

Contract Number: 35019

Starting Date: 06/14/73 Expiration Date: 06/13/76

Objective: Objective of this procurement is that progress in chemotherapy and in combined modality therapy be extended widely, utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults and hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors.

Proposed Course: The contractor will develop a prototype clinical chemotherapy program by recruitment of previously trained clinical chemotherapy fellows and by utilization of a system already established.

Progress: Have begun new outreach program in Pueblo. Lectures in radiotherapy. Set up tumor clinic in Pueblo. Continue to add patients with Hodgkin's disease to registry.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continuous care methods in leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $73,526

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. Myron Karon
4560 Sunset Boulevard
Los Angeles, California 90054

Performing Organization: School of Medicine, University of Southern California

Contract Number: 35013

Starting Date: 04/20/73 Expiration Date: 04/19/76

Objective: Objective of this procurement is that progress in chemotherapy and in combined modality therapy be extended widely, utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults and hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continuous care methods in leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $79,633

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. John Durant
University Station
Birmingham, Alabama 35294

Performing Organization: University of Alabama

Contract Number: 35014

Starting Date: 06/05/73 Expiration Date: 06/04/76

Objective: Objective of this procurement is that progress in chemotherapy and in combined modality therapy be extended widely, utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults and hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors.

Proposed Course: The contractor will develop a prototype clinical chemotherapy program by recruitment of previously trained clinical chemotherapy fellows and by utilization of a system already established.

Progress: Lymphoma network has been reviewed; treatment options for patients in the network are being discussed. Attention is being given to completion of system of documents for treatment of non-Hodgkin's lymphomas.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continuous care methods in leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $125,085

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. B. Lampkin
Elland and Bethesda Avenues
Cincinnati, Ohio 45229

Performing Organization: Children's Hospital Research Fund

Contract Number: 35015

Starting Date: 05/24/73 Expiration Date: 05/23/76

Objective: Objective of this procurement is that progress in chemotherapy and in combined modality therapy be extended widely, utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults and hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors.

Proposed Course: The contractor will develop a prototype clinical chemotherapy program by recruitment of previously trained clinical chemotherapy fellows and by utilization of a system already established.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continuous care methods in leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $77,281

CONTRACT SUMMARY

Title: Evaluation of Cancer Rehabilitation Systems to Improve Education

Principal Investigator: Dr. Carmella Gonnella
Office of the Treasurer
Atlanta, Georgia 30322

Performing Organization: Emory University

Contract Number: 45134

Starting Date: 06/25/74 Expiration Date: 06/24/77

Objective: To develop a program for the development and implementation of methodology for identifying, selecting, and evaluating comparable cancer rehabilitation systems, so that new demonstrable conceptualization of rehabilitation for oncology patients may be defined, and a new understanding of the procedure and roles of personnel involved in the rehabilitation of cancer patients; and development of new educational requirements which will improve the effectiveness of cancer patient rehabilitation.

Proposed Course: The contractor will develop a model program for rehabilitation of cancer patients based on data received from systems of target areas and expertise of consultant.

Progress: Accomplishments in staffing and contact with participating hospitals.

Significance for Cancer Control Program: To evaluate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $133,309

CONTRACT SUMMARY

Title: Development and Utilization of Rehabilitation and/or Continuing Care Resources and Services

Principal Investigator; Dr. Lack
765 Prospect Street
New Haven, Connecticut

Performing Organization: Hospice, Inc.

Contract Number: 55053

Starting Date: 09/03/74 Expiration Date: 12/02/75

Objective: To identify the resources, facilities and services needed in the outpatient clinic and at-home for the rehabilitation and/or continuing care of the oncology patient. Provide for the education of health care specialists. Evaluate the benefits of definite follow-up care. Contract will be extended for full three year period when costs for additional years can be determined accurately.

Proposed Course: The contractor will provide for continuing care by the organization of a multidisciplinary visiting health care team and volunteers.

Progress: The program has been extended to 60 families. Multidisciplinary team formed and volunteers trained.

Project Officer: Mr. Burke

FY 75 Funds: $331,762

CONTRACT SUMMARY

Title: Demonstration of Benefits of Early Identification of Psychosocial Problems, etc.

Principal Investigator: Dr. Theodore L. Phillips
Cancer Research Institute
San Francisco, California 94143

Performing Organization: University of California, San Francisco

Contract Number: 55190

Starting Date: 04/01/75 Expiration Date: 03/31/78

Objective: To develop a program to demonstrate the benefits of early identification of psychosocial problems and early intervention toward rehabilitation of cancer patients.

Proposed Course: The contractor will develop early identification criteria through utilization of standardized psychosocial tests and interviews. The program will be evaluated to determine the reliability and validity of the criteria.

Progress: Recruitment and selection of staff.

Project Officer: Mr. Burke

FY 75 Funds: $227,343

CONTRACT SUMMARY

Title: Demonstration of Benefits of Early Identification of Psychosocial Problems, etc.

Principal Investigator: Dr. John J. Dawson
151 East Bannock Street
Boise, Idaho 83702

Performing Organization: Mountain State Tumor Institute

Contract Number: 55189

Starting Date: 02/03/75 Expiration Date: 02/02/78

Objective: To develop a program to demonstrate the benefits of early identification of psychosocial problems and early intervention toward rehabilitation of cancer patients.

Proposed Course: The contractor will develop early identification criteria through utilization of standardized psychosocial tests and interviews. The program will be evaluated to determine the reliability and validity of the criteria.

Progress: Recruitment and selection of staff.

Project Officer: Mr. Burke

FY 75 Funds: $123,681

CONTRACT SUMMARY

Title: Demonstration of Benefits of Early Identification of Psychosocial Problems of Cancer Patients

Principal Investigator: Dr. Marcia Abramson
Iowa City
Johnson, Iowa 52242

Performing Organization: University of Iowa

Contract Number: 55061

Starting Date: 03/01/75

Objective: To develop a program to demonstrate the benefits of early identification of psychosocial problems and early intervention toward rehabilitation of cancer patients.

Proposed Course: The contractor will develop early identification criteria through utilization of standardized psychosocial tests and interviews. The program will be evaluated to determine the reliability and validity of the criteria.

Progress: Recruitment and selection of staff.

Project Officer: Mr. Burke

FY 75 Funds: $182,821

CONTRACT SUMMARY

Title: Modification of Employer's Attitude Toward Workable Cancer Patients

Principal Investigator: Dr. Martin Bloom
962 Wayne Avenue
Silver Spring, Maryland 20910

Performing Organization: Applied Management Sciences, Inc.

Contract Number: 55069

Starting Date: 04/10/75 Expiration Date: 04/09/78

Proposed Course: The contractor will undertake this project from an investigative empirical point of view. Information will be accumulated on attitudes of employment of workable patients from special attitude, management and behavior change skills.

Progress: Activities directed toward solution of management target areas. Phase I completed.

Project Officer: Mr. Burke

FY 75 Funds: $139,602

CONTRACT SUMMARY

Title: Modification of Employer's Attitude Toward Employment of Workable Cancer Patients

Principal Investigator: Dr. Melvin H. Rudov

Performing Organization: American Institute of Research

Contract Number: 55070

Starting Date: 01/08/75 Expiration Date: 01/07/78

Proposed Course: The contractor will undertake this project from an investigative empirical point of view. Information will be accumulated on attitudes of employment of workable patients from special attitude, management and behavior change skills.

Progress: Activities directed toward solution of management target areas.

Project Officer: Mr. Burke

FY 75 Funds: $149,027

CONTRACT SUMMARY

Title: Modification of Employer's Attitude Toward Employment of Workable Cancer Patients

Principal Investigator: Dr. Steinberg
5520 Wisconsin Avenue
Washington, D. C. 20014

Performing Organization: University Research Corporation

Contract Number: 55087

Starting Date: 12/31/74 Expiration Date; 06/30/77

Proposed Course: The contractor will undertake this project from an investigative empirical point of view. Information will be accumulated to attitudes of employment of workable patients from special attitude, management and behavior change skills.

Progress: Activities directed toward solution of management target areas. Phase I completed.

Project Officer: Mr. Burke

FY 75 Funds: $272,946

CONTRACT SUMMARY

Title: Modification of Employer's Attitude Toward Employment of Workable Cancer Patients

Principal Investigator: Dr. Wilson
300 N. Washington Street
Alexandria, Virginia 22314

Performing Organization: Human Resources Research Organization

Contract Number: 55089

Starting Date: 12/31/74 Expiration Date: 09/30/77

Proposed Course: The contractor will undertake this project from an investigative empirical point of view. Information will be accumulated on attitudes of employment of workable patients from special attitude, management and behavior change skills.

Progress: Activities directed toward solution of management target areas. Phase I completed.

Project Officer: Mr. Burke

FY 75 Funds: $168,923

CONTRACT SUMMARY

Title: Modification of Employer's Attitude Toward Workable Cancer Patients

Principal Investigator: Dr. Deeds
P. O. Box 1693
Baltimore, Maryland

Performing Organization: Westinghouse Electric

Contract Number: 55094

Starting Date: 11/11/74 Expiration Date: 11/10/77

Proposed Course: The contractor will undertake this project from an investigative empirical point of view. Information will be accumulated on attitudes of employment of workable patients from special attitude, management and behavior change skills.

Progress: Activities directed toward solution of management target areas. Phase I completed.

Project Officer: Mr. Burke

FY 75 Funds: $133,027

CONTRACT SUMMARY

Title: Enterostomal Therapy Education Program

Principal Investigator: Dr. M. Conway
881 Commonwealth Avenue
Boston, Massachusetts

Performing Organization: Boston University

Contract Number: 45112

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Develop new centers including course material, staff and facilities, for the education and training of enterostomal therapists. This contract to train a total of 105 therapists over duration of contract.

Proposed Course: The contractor will develop training aids and will design an intensive six-week course curricula for training of enterostomal therapists which will be concerned with the physical, psychological, social and economic needs of patients.

Progress: Curricula designed; recruitment procedure outlined; group of therapists has been accomplished.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in the colon and rectum.

Project Officer: Mr. Burke

FY 75 Funds: $85,428

CONTRACT SUMMARY

Title: Enterostomal Therapy Education Program

Principal Investigator: Dr. Hilkemeyer
M. D. Anderson Hospital
Houston, Texas 77025

Performing Organization: University of Texas

Contract Number: 45111

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Develop new centers including course material, staff and facilities, for the education and training of enterostomal therapists. This contract to train a total of 56 therapists, 16 in first year and 20 in each of the following two years of contract duration.

Proposed Course: The contractor will develop training aids and design an intensive six-week course curricula for training of enterostomal therapists which will be concerned with physical, psychological, social and economic aspects of the patient.

Progress: Curricula designed; applications have been accepted; first group of therapists prepared.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in the colon and rectum.

Project Officer: Mr. Burke

FY 75 Funds: $70,023

CONTRACT SUMMARY

Title: Enterostomal Therapy Training Program

Principal Investigator: Dr. William C. McGarity
Atlanta, Georgia 30322

Performing Organization: Emory University, Office of the Treasurer

Contract Number: 45102

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Develop new centers, including course material, staff and facilities, for the education and training of enterostomal therapists. Operate five six-week training courses each year and train a minimum of 30 persons each year.

Proposed Course: The contractor will develop training aids and design an intensive six-week course curricula for training of enterostomal therapists which will be concerned with the physical, psychological, social and economic needs of the patient.

Progress: The first group of enterostomal therapists has been trained; recruitment and acceptance for other groups has been accomplished.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in the colon and rectum.

Project Officer: Mr. Burke

FY 75 Funds: $64,297

CONTRACT SUMMARY

Title: Cancer Training Program for Physical/Occupational Therapists

Principal Investigator: Dr. John Healey, Jr.
6723 Bertner Avenue
Houston, Texas 77025

Performing Organization: University of Texas at Houston, M.D. Anderson Hospital

Contract Number: 45051

Starting Date: 05/01/74 Expiration Date: 04/30/76

Objective: Develop basic and advanced training course for physical/occupation therapists including new or revised curricula for education in special problems of cancer patients. Develop training materials and evaluation procedures and a model training program. Train 200 basic and 200 advanced students in two year period. Program is funded for one year at present.

Proposed Course: The contractor will develop a program to increase the awareness and preparation of physical therapy students in the management of the cancer patient through a basic preparatory course with integrated clinical training using training techniques.

Progress: The course has been designed; 15 participants have been trained; and 55 participants have been selected for classes through January. Courses and subsequent classes are scheduled.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $167,130

CONTRACT SUMMARY

Title: Training Program for Physical and/or Occupation Therapy

Principal Investigator: Dr. Nelson
310 East 35th Street
New York, New York 10016

Performing Organization: New York University

Contract Number: 45050

Starting Date: 12/03/73 Expiration Date: 12/02/75

Objective: Development, demonstration and evaluation of basic and/or advanced training curricula for physical/occupational therapists. Provide educational opportunities for students in physical/occupational therapy that will permit them to provide better care for oncology patients.

Proposed Course: The contractor will develop a program to increase the awareness and preparation of physical therapy students in the management of the cancer patient through a basic preparatory course with integrated clinical training using training techniques.

Progress: The contractor has developed a basic course and has prepared the first group of students according to this plan. Learning resources are to be developed.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $58,955

CONTRACT SUMMARY

Title: Training Program for Physical and/or Occupation Therapy

Principal Investigator: Dr. Kalish
1518 Clifton Road
Atlanta, Georgia 30322

Performing Organization: Emory University

Contract Number: 45045

Starting Date: 10/01/73 Expiration Date: 09/30/75

Objective: Development, demonstration and evaluation of basic and/or advanced training curricula for physical/occupational therapists. Provide educational opportunities for students in physical/occupational therapy that will permit them to provide better care for oncology patients.

Proposed Course: The contractor will develop a program to increase the awareness and preparation of physical therapy students in the management of the cancer patient through a basic preparatory course with integrated clinical training using training techniques.

Progress: Therapists have been trained beginning to enter other rehabilitation programs.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $105,716

CONTRACT SUMMARY

Title: Training Program for Physical and/or Occupational Therapy

Principal Investigator: Dr. Kent
Stanford, California 94305

Performing Organization: Stanford University School of Medicine

Contract Number: 45044

Starting Date: 10/01/73 Expiration Date: 09/30/75

Objective: Development, demonstration and evaluation of basic and/or advanced training curricula for physical/occupational therapists. Provide education opportunities for students in physical/occupational therapy that will permit them to provide better care on oncology patients.

Proposed Course: The contractor will develop a program to increase the awareness and preparation of physical therapy students in the management of the cancer patient through a basic preparatory course with integrated clinical training using training techniques.

Progress: Interview schedules for patients developed; curricula developed; final stages of curricula modification.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $125,811

CONTRACT SUMMARY

Title: Training of Professional Teams in Cancer Rehabilitation

Principal Investigator: Dr. John Healey, Jr.
M. D. Anderson Hospital and Tumor
Houston, Texas 77025

Performing Organization: The University of Texas System Cancer Center

Contract Number: 45038

Starting Date: 10/01/73 Expiration Date: 09/30/75

Objective: To establish a cancer rehabilitation personnel training program for the training of 10 to 12 professional teams from selected geographically distributed health institutions.

Proposed Course: The contractor will develop a model curriculum, short course training and (e.g., films, etc.) will select team instructors, will train teams of professional health personnel from geographically distributed regional health centers or large hospitals and will evaluate the effectiveness of the training course.

Progress: Curriculum has been designed. Rehabilitation teams and instructors have been selected. Contractor is now in evaluation stage.

Significance for Cancer Control Program: To demonstrate rehabilitative methods at multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $48,406

CONTRACT SUMMARY

Title: Integrated Cancer Rehabilitation Services

Principal Investigator: Dr. Thomas K. L. Lau
2230 Liliha Street
Honolulu, Hawaii 96817

Performing Organization: Saint Francis Hospital

Contract Number: 45118

Starting Date: 06/30/74 Expiration Date: 06/29/76

Objective: Develop model patient rehabilitation service system providing medical, psychological and social support covering entire course of patient's illness. Establish comprehensive integrated approach to needs of cancer patients, their families, and the community. Establish and implement staff training methods and coordinate actions of various departments and disciplines, leading to similar programs in other hospitals.

Proposed Course: The contractor will focus on three areas of programming: (1) development of a comprehensive integrated system, (2) establishment of a new health professional, and (3) evaluation.

Progress: Referral procedures devised; orientation of staff; roles of team members receiving clarification.

Significance for Cancer Control Program: To field test rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $192,127

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. Guy Robbins
444 East 68th Street
New York, New York 10021

Performing Organization: Memorial Hospital for Cancer and Allied Diseases

Contract Number: 55223

Starting Date: 03/06/75 Expiration Date: 03/05/76

Objective: To develop a model and workable system to provide rehabilitation services to cancer patients. Develop educational programs for personnel needed to implement such a system. Provide such educational programs on a demonstration basis and for a limited time period.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary team approach, for cancer patients and their families from diagnosis through follow-up.

Progress: Total implementation of operational procedures, production of important manuals and preparation of pilot audio-visual presentations related to rehabilitation training.

Project Officer: Mr. Burke

FY 75 Funds: $452,222

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. Phala A. Helm
5323 Harry Hines Boulevard
Dallas, Texas 75235

Performing Organization: University of Texas Health Science Center

Contract Number: 45133

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To develop model patient rehabilitation service system providing medical, psychological and social support covering entire course of a patient's illness. Demonstrate and report on effective techniques for rehabilitative care. Provide continuing education of basic rehabilitation team and inform other staff of new rehabilitative techniques.

Proposed Course: The contractor will establish, through a six-phase plan, a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary approach, for cancer patients and families from diagnosis through follow-up.

Progress: Contractor is presently involved in Phase II - Education and Promotion and Phase III - Implementation.

Project Officer: Mr. Burke

FY 75 Funds: $133,309

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. A. I. Sutnick
7701 Burholme Avenue
Fox Chase, Pennsylvania 19111

Performing Organization: Institute for Cancer Research

Contract Number: 45126

Starting Date: 06/15/74 Expiration Date: 06/14/77

Objective: Develop and implement the concept of a model rehabilitation team specific to oncology patients. Service must provide medical, psychological, and social support covering entire period of a patient's illness. Demonstrate and report on effective rehabilitation techniques developed. Provide continuing education of team members and in-house staff. Evaluate the progress and effectiveness of the program.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary approach, for cancer patients and their families from diagnosis through follow-up.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $477,617

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and Departments

Principal Investigator: Dr. O. H. Beahrs
200 First Street, SW
Rochester, Minnesota 55901

Performing Organization: Mayo Foundation

Contract Number: 45120

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Develop model patient rehabilitation service system providing medical, psychological and social support covering entire course of patient's illness. Implement educational and instructional programs for each discipline and include staff of affiliated organizations in educational programs.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary approach, for cancer patients and their families from diagnosis through follow-up.

Progress: Accomplishments in staff development and materials development (i.e., brochures, audio-visuals) and extramural programming.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $207,368

CONTRACT SUMMARY

Title: Integrated Cancer Rehabilitation Services

Principal Investigator: Dr. Barber
89th Avenue and Van Wyck Expressway
Jamaica, New York 11418

Performing Organization: The Jamaica Hospital

Contract Number: 45034

Starting Date: 08/30/73 Expiration Date: 08/29/76

Objective: The contractor shall develop a model patient rehabilitation service system that will provide the medical, psychological and social support required to return the cancer patient to a normal and productive life. The cancer rehabilitation system to be established at a comprehensive medical care center, should cover the entire course of a patient's illness, starting from the diagnosis of cancer and continuing through treatment and all necessary follow-up support after discharge.

Proposed Course: The contractor will focus on three major areas of programming: (1) development of a comprehensive integrated system, (2) establishment of a new health professional, (3) evaluation.

Progress: Staff recruitment; curricula development; begun patient contact and coordination of services.

Significance for Cancer Control Program: To field test rehabilitative methods at multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $193,273

CONTRACT SUMMARY

Title: Demonstration of Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. Guy F. Robbins
444 East 68th Street
New York, New York 10021

Performing Organization: Memorial Hospital for Cancer and Allied Disease

Contract Number: 45052

Starting Date: 12/01/73 Expiration Date: 11/30/74

Objective: To develop a model and workable system to provide rehabilitation services to cancer patients. Develop educational programs for personnel needed to implement such a system. Provide such education programs on a demonstration basis and for a limited time period.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary team approach, for cancer patients and their families from diagnosis through follow-up.

Progress: Total implementation of operational procedures, production of important manuals and preparation of pilot audio-visual presentations related to rehabilitation training.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $338,000

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. Lehmann
1959 N. E. Pacific Avenue
Seattle, Washington 98105

Performing Organization: University of Washington

Contract Number: 45039

Starting Date: 04/01/74 Expiration Date: 03/31/77

Objective: Develop model patient rehabilitation service system providing medical, psychological and social support covering entire course of patient's illness. Demonstrate and report on effective techniques for rehabilitative care. Provide continuing education for rehabilitation team members. First phase consists of planning, second phase implementation, third phase evaluation and revision. Each phase expected to require year's work.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary team approach, for cancer patients and their families from diagnosis through follow-up.

Progress: Basis for evaluation and implementation of a model rehabilitation service referral delivery system to cancer patients has been established. Screening protocol established.

Significance for Cancer Control Program: To demonstrate rehabilitative methods in multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $244,634

CONTRACT SUMMARY

Title: Demonstration of Cancer Rehabilitation Facilities and/or Departments

Principal Investigator: Dr. Ben V. Branscomb
University Station
Birmingham, Alabama 35294

Performing Organization: University of Alabama

Contract Number: 35032

Starting Date: 06/29/73 Expiration Date: 06/29/75

Objective: The contractor shall develop a model patient rehabilitation service system that will provide the medical, psychological and social support required to return the cancer patient to a normal and productive life. The cancer rehabilitation system, to be established at a comprehensive medical care center, should cover the entire course of a patient's illness, starting from the diagnosis of cancer and continuing through treatment and all necessary follow-up support after discharge.

Proposed Course: The contractor will establish a coordinated educational and rehabilitation service program, whose integral aspect involves a multidisciplinary team approach, for cancer patients and their families from diagnosis through follow-up.

Progress: Model service system is well established with a trained and stable group of personnel.

Significance for Cancer Control Program: To demonstrate rehabilitative methods at multiple sites.

Project Officer: Mr. Burke

FY 75 Funds: $366,025

CONTRACT SUMMARY

Title: Prototype Clinical Chemotherapy in Cancer Control

Principal Investigator: Dr. O. Ross McIntyre
Dartmouth College
Hanover, New Hampshire 03755

Performing Organization: Dartmouth Medical School, Dartmouth College

Contract Number: 35017

Starting Date: 06/05/73 Expiration Date: 06/04/76

Objective: Objective of this procurement is that progress in chemotherapy and in combined modality therapy be extended widely, utilizing existing centers with the ability to bring modern combined modality therapy to susceptible tumors in children and/or adults and hospitals with the ability to provide high quality combined modality therapy for one or more of the susceptible tumors. Year 2 funding added 4/14/74.

Proposed Course: The contractor will develop a prototype clinical chemotherapy program by recruitment of previously trained clinical chemotherapy fellows and by utilization of a system already established.

Progress: Cooperative agreement for project participants has been developed. Protocol for staging and treatment of previously untreated malignant lymphoma has been submitted.

Significance for Cancer Control Program: To demonstrate the treatment, follow-up and continous care methods in leukemia and lymphoma.

Project Officer: Dr. Hamner

FY 75 Funds: $44,661

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Cancer Centers

Principal Investigator: Dr. Norma Kolthoff
Professor of Nursing, University of Wisconsin
1402 University Avenue
Madison, Wisconsin 53706

Performing Organization: University of Wisconsin

Contract Number: 45115

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Develop and implement in NCI supported cancer centers comprehensive training programs for nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Project Officer: Dr. Conley

FY 75 Funds: $119,716

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Cancer Centers

Principal Investigator: Ms. Mary Brown
1275 York Avenue
New York, New York 10021

Performing Organization: Memorial Hospital for Cancer and Allied Diseases

Contract Number: 45123

Starting Date: 06/01/74 Expiration Date: 05/31/77

Objective: Develop and implement in NCI supported cancer centers comprehensive training programs for nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: To demonstrate treatment, follow-up and continuous care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $147,684

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Cancer Centers

Principal Investigator: Ms. Renilda Hilkemeyer
Director, Nursing Service, M. D. Anderson Hospital
6723 Bertner Avenue
Houston, Texas 77025

Performing Organization: University of Texas System Cancer Center

Contract Number: 45127

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Develop and implement in NCI sponsored cancer centers comprehensive training programs for nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: To demonstrate treatment, follow-up and continuous care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $262,334

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Cancer Centers

Principal Investigator: Dr. Edna L. Fritz
Director, School of Nursing, Ohio State University
1585 Neil Avenue
Columbus, Ohio 43210

Performing Organization: Ohio State University Research Foundation

Contract Number: 45128

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Develop and implement in NCI-sponsored cancer centers comprehensive programs for training nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: To demonstrate treatment, follow-up and continuous care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $251,404

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Medical Centers

Principal Investigator: Ms. Donna Diers, Dean
School of Nursing, Yale University
38 South Street
New Haven, Connecticut 06519

Performing Organization: Yale University

Contract Number: 45138

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: Develop and implement in medical centers comprehensive training programs for nurses in specialized techniques and practices used in cancer patient care. Anticipate 250 students trained on this contract.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects to treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Project Officer: Dr. Conley

FY 75 Funds: $246,495

CONTRACT SUMMARY

Title: Oncology Training Programs in Medical Centers

Principal Investigator: Ms. M. H. Walton
Associate Professor and Assistant Dean
College of Nursing, University of Utah
Salt Lake City, Utah 84112

Performing Organization: University of Utah

Contract Number: 45141

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To develop and implement in medical centers comprehensive training programs for nurses in specialized techniques and practices used in cancer patient care. Anticipate 269 students trained on this contract.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: To demonstrate treatment and follow-up care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $250,939

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Community Hospitals

Principal Investigator: Dr. Carl W. Boyer, Jr.
Queen's Medical Center
P. O. Box 861
Honolulu, Hawaii 96808

Performing Organization: Queen's Medical Center

Contract Number: 45153

Starting Date: 06/28/74 Expiration Date: 06/27/77

Objective: To develop and implement in community hospitals comprehensive training programs for nurses in specialized techniques and practices used in cancer patient care. 250 nurses to be trained in three years under this contract.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Project Officer: Dr. Conley

FY 75 Funds: $92,616

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Medical Centers

Principal Investigator: Ms. Dorothy T. Wilson
666 Elm Street
Buffalo, New York 14203

Performing Organization: New York State Department of Health, Roswell Park Division

Contract Number: 55063

Starting Date: 09/01/74 Expiration Date: 08/31/77

Objective: To develop and implement in NCI-supported cancer centers comprehensive programs for training nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instrucitonal media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: Treatment and follow-up care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $90,388

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Cancer Centers

Principal Investigator: Ms. Doris Cayer
Director, Department of Continuing Education
Boston University School of Nursing
635 Commonwealth Avenue
Boston, Massachusetts 02215

Performing Organization: Boston University

Contract Number: 55142

Starting Date: 10/15/74 Expiration Date: 10/14/77

Objective: To develop and implement in NCI-supported cancer centers comprehensive programs for training nurses in the specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for the Cancer Control Program: Treatment and follow-up care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $117,179

CONTRACT SUMMARY

Title: Oncology Nursing Programs In Community Hospitals

Principal Investigator: Ms. Alice J. Labadie
1120 South Utica Avenue
Tulsa, Oklahoma 74104

Performing Organization: Hillcrest Medical Center

Contract Number: 55181

Starting Date: 10/01/74 Expiration Date: 09/30/77

Objective: To develop and implement in community hospitals comprehensive training programs for nurses in specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: Treatment and follow-up care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $72,874

CONTRACT SUMMARY

Title: Oncology Nursing Programs in Community Hospitals

Principal Investigator: Ms. Charlotte C. Wright
64 Robbins Street
Waterbury, Connecticut 06720

Performing Organization: Waterbury Hospital Health Center

Contract Number: 55185

Starting Date: 06/28/74 Expiration Date: (In process of renegotiation)

Objective: To develop and implement in community hospitals comprehensive training programs for nurses in specialized techniques and practices used in cancer patient care.

Proposed Course: Initiate and develop new educational programs, or expand existing programs for nursing personnel. At least one course should be a basic "core" course of two weeks (80 hours). Longer continuing education courses may be provided. At least one course should make provisions for Licensed Practical Nurses and/or Licensed Vocational Nurses. The program will emphasize the nursing aspects of treatment, rehabilitation and long-term care of cancer patients as well as nursing responsibilities in prevention, screening, detection and supportive care of patients and families.

Progress: The courses are being offered as scheduled with a program design using educational methods and instructional media consistent with the background and experience of the trainees. Recruitment of trainees is in process. Appropriately qualified faculty have been recruited. Program evaluation is an integral part of the program design.

Significance for Cancer Control Program: To demonstrate treatment and follow-up care methods in multiple sites.

Project Officer: Dr. Conley

FY 75 Funds: $225,000

SUPPORTIVE SERVICES

Controlling cancer is not only a responsiblity of the medical profession. It requires the involvement of a variety of specialties which can help ensure that what research has discovered is applied to the broadest possible segment of society.

The Supportive Services activity assists the Intervention and Community Activities programs by providing those services which are common to both and, beyond that, which complement their efforts by supporting the transition between field tests and demonstrations to more wide-spread community-level projects.

The Supportive Services activity is composed of the Planning, the Evaluation, the Resources, the Communications, and the Liaison Branches. Each has a distinct responsibility not only for its own programs but also to make certain their activities extend the reach of demonstration programs to the entire nation.

Since the creation of Supportive Services in mid-FY 75, the primary effort has been to develop comprehensive plans for providing the widest possible range of services. While this planning process was underway, the separate Branches continued to implement projects which, in the aggregate, have long-range implications for Intervention and Community Activities programs. For example, the Resources Branch sponsored the first conference on the Behavioral Aspects of Cancer Control ever held. The resulting inquiry will support Intervention programs by providing a better understanding of the human considerations in cancer control. Meanwhile, the Communications Branch set up Cancer Control Communications offices in each of the Comprehensive Cancer Centers which will ultimately provide intervention and community activities programs with immediately responsive communication and promotion services on a regional basis.

The continuing concern of Supportive Services is to offer a multidimensional approach to the application of cancer knowledge which can enlarge the impact of the Intervention programs far beyond the limits of a particular contract or grant.

EVALUATION BRANCH

Measuring the impact of the Division's intervention programs on reducing the incidence, morbidity and mortality from cancer requires accurate and timely data collection and evaluation. During FY 75, the Evaluation Branch provided the Intervention activity with consultative services based on three primary approaches to meeting the near-term needs of the Division.

First, much evaluation focused on the effect Intervention programs had on reducing the severity of cancer by earlier detection, especially through the introduction of new detection techniques. One such evaluation determined that the use of mammography, thermography and clinical examinations could improve upon the early detection rate of breast cancer by over 25 per cent.

Secondly, on the assumption that the maximum impact of any Intervention program is possible only if all program components are functioning correctly, the Branch engages in process evaluation. Each component of an Intervention program is closely examined for efficiency and its effect on all other aspects of the program.

Lastly, the Branch has attempted to build into each Intervention project a self-evaluation capability which will continue to provide necessary data once federal funding of the project has ended.

Specific activities in support of the Intervention activity during FY 75 have included development of a unit screening report for each woman examined in the State Screening Program for Carcinoma of the Cervix. Analysis of the reports during FY 76 will reveal whether the program is reaching those women at greatest risk to cervical cancer.

Common data collection forms were designed for the Head and Neck Cancer Management Demonstration Network. This evaluation will detail how effective an interdisciplinary approach has been in the early detection, diagnosis and treatment of head and neck cancer.

In developing evaluation plans for the asbestos and vinyl chloride projects, the Division's major FY 75 effort to monitor the effects of carcinogenic environments on industrial workers, the Evaluation Branch constructed a more general occupational exposure model applicable to most industries. The Evaluation Branch also assisted the Rehabilitation Branch in designing a survey to discover attitudes and practices of employers in hiring or reinstating work-able cancer patients.

During FY 75, the Branch issued several RFPs for the accumulation of baseline data on cost of cancer care, evaluation of cervical screening programs, patient attitudes and knowledge about cancer control and personnel required for cancer control. Final disposition of these RFPs occurred in late FY 75, with the latter two studies being transferred to the Resources Branch for implementation and monitoring.

CONTRACT SUMMARY

Title: Data Management Center for Breast Cancer Demonstration Program

Principal Investigator: Dr. Perry A. Scheinok
3508 Science Center
Philadelphia, Pennsylvania 19104

Performing Organization: University City Science Center

Contract Number: 45161

Starting Date: 06/28/74 Expiration Date: 06/27/75

Objective: This contract is a successor to CN 35035 and is a continuation of that project. UCSC to serve as coordinator to collect and summarize data from 27 participating clinics. Each to screen 5,000 women per year for two years. Maintain data processing center and patient register, develop statistical analysis techniques

Significance for Cancer Control Program: To evaluate screening and detection techniques of the female breast.

Project Officer: Drs. Pomerance and Cutler

FY 75 Funds: $46,000

RESOURCES BRANCH

The materials, manpower and facilities needed to keep a nation provided with the latest cancer control knowledge must be identified and developed. The Resource Branch gives the Division the capability to address the training and education needs which emerge from the Division's intervention demonstration programs and to prepare strategies for acquiring the necessary facilities and materials.

During FY 75, the Branch concentrated on providing support for professional education, public education and research oriented toward obtaining a better understanding of the behavioral factors in cancer control.

In providing services to professional education, the Branch prepared catalogs of cancer materials for cytotechnologists and nurses and updated the manual of cytotechnology, first published in 1962.

A project to develop curricula guidelines relevant to cancer for dental schools grew out of a survey of progress in cancer education conducted under contract by the American Cancer Society. Similar plans have been made to provide guidelines to schools of nursing and social work.

A Branch-sponsored conference on the behavioral aspects of cancer probed the human factors involved in delay in seeking medical advice and diagnosis, failure of the public to heed warnings about cancer and effect of cancer diagnosis on patients and their families. The first of its kind ever held, the Conference featured several presentations which are being compiled into book form so that the human side of cancer control can become a part of cancer treatment. Psychosocial consideration in cancer treatment will continue to occupy the Branch as this area of concern becomes more relevant to rehabilitation and continuing care.

Public education has involved the Branch in making cancer knowledge available to the general public via toll-free telephone. Application of findings following evaluation of the initial efforts will assist the Communications Branch in implementing the nationwide "ControLine" service of toll-free response from the 17 Comprehensive Cancer Centers during FY 76.

The Branch is directing a special effort to provide Blacks, Spanish Americans and American Indians with cancer information. Small target audiences within these groups will be used to test various ways of presenting the information. Ultimately, the most effective approaches will be used to reach larger audiences. This technique was also used to determine the quality of public education on cancer. This study was undertaken so non-Federal public and private organizations can base their public education efforts on proven methods.

The Branch is also seeking information on the impact of the diagnosis of breast cancer in order to provide professionals with knowledge of their patients' concerns so better care can be provided.

CONTRACT SUMMARY

Title: National Cancer Consultative Programs for Hospitals

Principal Investigator: Mayer
55 East Erie Street
Chicago, Illinois 60611

Performing Organization: American College of Surgeons

Contract Number: 35030

Starting Date: 06/25/73 Expiration Date: 06/30/74

Objective: Review and approval of hospital-based cancer programs. Advice, assistance on program and registry activity. Sponsorship of geographic liaison fellows meetings, workshop and continuing education for hospital professionals. Revision of manuals for cancer programs and follow-up of patients. Renewed June 30, 1974 - June 30, 1975 for $197,486.

Proposed Course: Inspection of facilities and records support decision to provide approval by hospital category. Advice and assistance are provided for registering various meetings and carried out to provide continuing education.

Progress: Progress report has been requested.

Significance for Cancer Control Program: To promote general program support at multiple sites.

Project Officer: Dr. Nelson

FY 75 Funds: $200,000

CONTRACT SUMMARY

Title: Review and Evaluation Methods of Public Education in Cancer

Principal Investigator: Dr. Paisley
Institute for Community Relations
Stanford, California 94305

Performing Organization: Stanford University

Contract Number: 45041

Starting Date: 10/24/73 Expiration Date: 12/13/74

Objective: The purpose of this contract is to identify cancer public information and education programs which have achieved their objectives with reasonable expenditures, and to analyze the results in terms of methodology. Modified 10/24/74; expiration date 12/31/74; additional $7,465.

Proposed Course: Contractor has visited various sites where public education is taking place. Has interviewed considerable numbers of personnel and has evaluted the programs which are in existence.

Progress: Computer print-out on which the final report will be based has been received.

Significance for Cancer Control Program: To identify and evaluate preventive methods, screening and detection techniques in multiple sites.

Project Officer: Dr. Recer

FY 75 Funds: $7,465

CONTRACT SUMMARY

Title: Telephone Cancer Public Information Services

Principal Investigator: Dr. Mirand
666 Elm Street
Buffalo, New York 14203

Performing Organization: New York State Department of Health

Contract Number: 45073

Starting Date: 04/01/74 Expiration Date: 03/31/76

Objective: Expand the can-dial system of cancer information for the public to cover a 24-hour period and extend throughout the weekend. This requires the preparation of additional tapes and purchase of additional equipment. An advisory committee to be appointed to advise and evaluate. A program of liaison and information exchange should be instituted between sponsoring agencies and community groups. Bank of tapes updated and equipment used for replay. Dissemination of information should be expanded. Based on unsolicited proposal.

Significance for Cancer Control Program: To evaluate preventive methods, screening and detection techniques in multiple sites.

Project Officer: Dr. Recer

FY 75 Funds: $120,187

COMMUNICATIONS BRANCH

Cancer Control knowledge is communicated daily by many private and Federal efforts. It is the plan of the Communications Branch to further extend the reach of ongoing efforts while speeding new facts developed in DCCR projects to health professionals and the public they serve.

To make this happen, a 3-year regionalized plan has been developed to increase the channels of communication, link those channels into a network capable of rapid transmission to every community and to coordinate other private and Federal effort, so that the maximum amount of information is presented with a unity of theme.

Steps toward implementing this plan were taken when regional Cancer Control Communications programs were established in the 17 Comprehensive Cancer Centers. Funds were provided through contracts to support the establishment of core staffs of communications offices and to finance the development of materials and resources required to extend the control program.

The highest priority activity for these offices during FY 75 was the installation of toll-free WATS systems so the public may call for answers to any question about cancer. The Communications Branch contracted directly with AT&T to provide this WATS service to all 17 communications programs to ensure that the system will be fully operational in all 50 states during FY 76. A model training program for volunteers and others has been developed by the Communicatons Branch and will be offered to these regional communications offices so a high quality service can be maintained. Following training of the volunteers, an intensive promotional program will acquaint the public with the service.

The establishment of the Cancer Control Communications Network begins a process by which the proven results of the Division's intervention programs can be promoted and implemented without requiring additional Federal funding. Promotion, when supported by the outreach activities of the regional Cancer Control Communications programs, will stimulate communities to call upon other programs of the Division for technical assistance in organizing community-level cancer control programs. Response from the Division to these community inquiries will come primarily from the Resources Branch and from the Community Activities program. Thus, there is a sequential method for making certain the intervention knowledge generated by the Division's demonstration program is widely applied.

Once the network is completed, the Communications Branch can respond immediately to the needs of Division-generated activities for communications services. Regional Communications Offices can be directed to assist in preparing materials which will enable branch projects to communicate more effectively. Field test of this method of support to DCCR projects already is underway on a limited basis in the Texas asbestos workers program.

Discussions continue with the American Cancer Society for collaborative efforts in which the Communications Branch will provide consultation and technical assistance to ACS activities related to programs of the National

Cancer Program. The Communications Branch is also collaborating with the Office of Cancer Communications, NCI, in creating a mutual information clearinghouse for cancer communications. This clearinghouse will be the central collection and distribution point to assist those answering public inquiries about cancer.

Among the innovative activities planned by the Communications Branch in cooperation with the Community Activities Branch is the organization and conducting of prototype community-level programs to communicate the value of cancer screening for early detection.

CONTRACT SUMMARY

Title: Comprehensive Cancer Center Communications Network

Principal Investigator: Dr. Gerald P. Murphy
Roswell Park Division
Buffalo. New York 14203

Performing Organization: New York State Department of Health and Health Research, Inc.

Contract Number: 55174

Starting Date: 05/01/75

Objective: To serve as a focal point within the center for information about cancer for the public and health professionals.

Proposed Course: The contractor will establish an information/education office and, by the end of the first year, install WATS lines for hotline services for the public. Contractor will conduct the program, including promotion for a total of three years.

Progress: Recruiting personnel to establish office.

Project Officer: Mr. Rhone

FY 75 Funds: $83,223

CONTRACT SUMMARY

Title: Comprehensive Cancer Center Communications Network

Principal Investigator: Dr. Harold P. Rausch
750 University Avenue
Madison, Wisconsin 53706

Performing Organization: Board of Regents of the University of Wisconsin System

Contract Number: 55228

Starting Date: 06/01/75

Objective: To serve as a focal point within the center for information about cancer for the public and health professionals.

Proposed Course: The contractor will establish an information/education office and, by the end of the first year, install WATS lines for hotline services for the public. Contractor will conduct the program, including promotion for a total of three years.

Progress: Recruiting personnel to establish office.

Project Officer: Mr. Rhone

FY 75 Funds: $80,895

CONTRACT SUMMARY

Title: Comprehensive Cancer Center Communications Network

Principal Investigator: Dr. Alfred L. Frechette
35 Binney Street
Boston, Massachusetts 02115

Performing Organization: Sidney Farber Cancer Center

Contract Number: NO1-CN-55229

Starting Date: 06/30/75

Objective: To serve as a focal point within the center for information about cancer for the public and health professionals.

Proposed Course: The contractor will establish an information/education office and, by the end of the first year, install WATS lines for hotline services for the public. Contractor will conduct the program, including promotion for a total of three years.

Progress: Ready to recruit personnel.

Project Officer: Mr. Rhone

FY 75 Funds: $90,282

LIAISON BRANCH

The chief objective of the Liaison Branch is to establish and maintain for DCCR an effective exchange of information on matters relating to cancer control with other government agencies, national professional and voluntary health organizations, state and local health agencies, concerned industries and labor unions, etc.

Since establishment of the Branch in October 1974, a good start has been made in developing cooperative relationships with the many health service delivery programs of the Health Services Administration and with the Bureau of Health Manpower, the National Center for Health Statistics, and the National Center for Health Services Research in the Health Resources Administration. Awaiting agreement on a date is a meeting with the Director of the new Bureau of Health Planning and Resources Department.

The Division has had a number of contacts with SRS to develop better cancer detection programs in rural areas. The Liaison Branch also participated in the development of guidelines for the Early Periodic Screening, Detection and Treatment (EPSDT) Program administered by SRS for beneficiaries under age 21 of Titles V, XVIII and XIX. The guidelines were developed by a task force under the Division of Clinical Services of the Health Services Administration.

Liaison activities are also carried out by other branches of DCCR. The Prevention Branch is working in cooperation with the National Institute for Occupational Safety and Health, the National Center for Health Education, the National Clearinghouse for Smoking and Health of the Center for Disease Control, and with the Occupational Safety and Health Administration of the Department of Labor. The Resources Branch works with the Association of American Medical Colleges, the American Medical and Dental Associations, the American Association for Cancer Education, the National League for Nursing, the American Nurses Association, etc.

A high priority is placed on achievement of a two-way flow of information with appropriate national professional organizations to help them develop or increase the efforts of their members in improving detection, diagnosis and treatment of cancer and the rehabilitation and continuing care of patients with the disease. During the past year, cooperative efforts have been started with the American College of Obstetrics and Gynecology which has established task forces on pelvic and breast cancers; with the American College of Radiology in programs to improve the quality of mammography in the U. S.; with the American College of Surgeons to continue and improve its evaluation and approval of hospital cancer programs and to obtain the help of its Liaison Fellows in promoting cancer control activities; with the American Academy of Family Practice to help in its efforts to improve the participation of its members in cancer detection, diagnosis, rehabilitation and continuing care.

Finally, the Liaison Branch is working toward the establishment of a file for the DCCR of up-to-date information on the goals, organizational framework, officers, meetings and relevant committees and activities of all national professional and voluntary health organizations and specialty societies which are concerned with cancer control and arranging for the regular exchange of information of interest.

COMMUNITY ACTIVITIES PROGRAM

The objective of the Community Activities Program is to assist in the application of methodologies and techniques for mobilizing community resources to implement cancer control programs which can serve as models for replication nationally.

These demonstration programs are based on intervention procedures previously field tested by the Division or proven elsewhere. They are supplied to selected communities which have undertaken the necessary organizational planning activities. Such large-scale demonstrations require stimulation of the community at large to accept and prepare for the program and continuing consultation in the form of monitoring and evaluation.

Activating the community-based saturation program has been the principal effort of this activity since its creation in January 1975. Funds were provided to nine communities with acceptable tumor registry systems compatible with the SEER (Surveillance Epidemiology and End Results) Programs for planning and to two communities for 5-year program implementation.

Two branches within the Community Activities Program were established near the end of FY 75: the Community Resources Branch, which is responsible for the development of the community-based saturation program, and the Special Projects Branch, which becomes involved in specific community outreach programs conducted by Comprehensive Cancer Centers or multiprotocol organizations.

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